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A BACTERIAL DISEASE OF THE MANGO. *BACILLUS MANGIFERAE* N.SP.

BY ETHEL M. DOIDGE, M.A., F.L.S.,

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(*Thesis approved for the Degree of Doctor of Science in the University of the Cape of Good Hope.*)

(With Plates I—XIV and 3 Text-figures.)

FOR some years past, fruit growers in certain districts of this country have been troubled by a disease of mangoes which is causing considerable havoc in their orchards and threatens to seriously affect the export trade.

All parts of the tree are attacked, the affected areas becoming discoloured, while in fruit and stem deep longitudinal cracks also occur; the disease has been described by farmers as resembling in appearance the anthracnose of the grape vine caused by *Gloeosporium ampelophagum* Sacc., and its external effects are not dissimilar to those caused by that fungus. A large percentage of the fruit falls to the ground whilst yet immature, and the mangoes which remain on the trees are rendered unsightly and unfit for the market.

The disease was first reported in December, 1909, as occurring at Barberton in the Transvaal, and as being unchecked in its progress by repeated applications of Bordeaux mixture. It was soon found that most of the mango-growing districts of the Union were similarly affected and it was considered advisable to make a more detailed study of the disease and the organism causing it, with a view to formulating, if possible, some method by which its progress might be checked. This purpose has not yet been fully carried out as no effective remedy has been found, but the work has progressed to a point where it seems advisable that the results which have been obtained should be placed on record.

Literature.

There is no description in available literature of a disease of mangoes in any way resembling the one under discussion. General literature on the mango is very scanty and consists for the most part of pamphlets describing varieties and methods of propagation. In certain of these, however, reference is made to the diseases to which the mango is subject, and a short review of such references may not be out of place here.

Three diseases are reported from Hawaii (7), a blight caused by *Colletotrichum* sp.; mango scab, and sooty mould. In Hawaii and Cuba the bloom blight due to *Gloeosporium mangiferae* is common, attacking the opening blossoms and causing them to turn black, dry up and fall (3 and 6). This disease appears to be common in most districts where the mango is cultivated and has been reported in this country from Natal.

There is no reference to any fungous or bacterial trouble in a pamphlet entitled *The Mango, its Culture and Varieties*, by C. Marshall Woodrow (12), although remedies are suggested against certain insect pests in a paragraph on the "Enemies of the Mango Tree."

"The mango in Porto Rico seems almost entirely free from disease, or the attacks of insects. On the north side of the island the skin of the fruit is frequently disfigured by black spots, probably a fungus. Though in no way injuring the eating quality of the fruit, these detract from its appearance....In drier localities this discolouration was not observed" (4).

"Some of the varieties of mangoes cultivated in Trinidad are liable to a disease which makes the interior of the fruit assume a dark, jelly-like consistency, and the pulp immediately surrounding the seed becomes completely sour and uneatable. This does not arise from being over-ripe, as the same state of things may be seen in a half-ripe mango if cut. Many of the trees in the Botanic Gardens are affected, and hitherto we have been unable to ascertain the immediate cause."

"It is curious to note, however, that in some cases individual trees only are affected, while others growing near by produce perfectly sound fruit. It is certainly more prevalent with the better class of fruit than with the commoner kinds, but it is to be seen in all more or less. Again, some of the fruit of a tree may be perfectly sound, while another portion just as carefully handled will be utterly useless" (13).

There is no mention of any disease in a paper on "The Mango in South California" (9).

Of the six diseases mentioned above, four are due to the attacks of fungi; the remaining two, of which the cause has not been determined, differ essentially in their effects from the one to be described.

Geographical distribution.

Our attention was first drawn to this disease by Messrs Winter Bros. of Barberton, who sent specimens of mangoes from their trees for examination. They stated that it made its first appearance there after the great hail storm in October 1906, which damaged the trees considerably; the marks of the hail are still visible on the trunks and larger branches. A tree standing in a corner of the orchard was the first to become infected, and from this point the infection rapidly spread with the prevailing winds until every tree was diseased. In 1908, in spite of repeated spraying with Bordeaux mixture, they obtained not a single fruit from 60 trees. Almost all the fruit in the town was similarly affected, but at the farms, some distance away, the fruit was then perfectly sound. Every season up to the present (1913–14) the disease has been steadily gaining ground, each year spreading to some orchard which was previously free from infection, and this in spite of the fact that there have been four very dry seasons in succession, a condition which—as will be shown later—is highly unfavourable to the spread of the disease.

In 1910, a sample of infected fruit was received from Warmbaths. On making an inspection of the orchard in which the fruit was grown, it was found that the infection had started at a corner of the orchard where some young trees had been planted. These young trees were badly infected, and, as at Barberton, the infection had spread with the prevailing winds until a large proportion of the trees was affected. The trees which were apparently the original source of infection had been purchased in Natal, and it seemed probable that in this case the disease had been introduced from that province.

The writer visited Natal in March 1910, with a view to discovering whether this was the case, and found that it was prevalent throughout the mango-growing districts of Natal. It was actually observed on the leaves and stems of trees at Hillarys, Malvern and Durban. During a later visit in December 1911, it was seen on the fruit in all these localities.

In 1910, Mr R. A. Davis, the Government Horticulturist, reported that he had examined trees in Swaziland and found them quite healthy. These trees had all been imported from Natal some 14 years previously,

and this would seem to point to the fact that the disease was not known in Natal at that time. This idea was confirmed by a statement made by Dr Medley Wood, who was, until recently, Director of the Durban Botanic Gardens, and who informed me that whereas some years back he sold mangoes from the trees in the gardens up to the value of £30, recently he had scarcely obtained sufficient for his own use.

In August, 1911, an inspection of the mango trees at the Tzaneen and Westphalia Estates in the Zoutpansberg District showed them to be quite free from this trouble.

This disease was noted on some mango fruits from Lourenço Marques in November, 1910; but in Portuguese East Africa would appear to be confined to the neighbourhood of Delagoa Bay; for in a letter dated 14th November, 1913, Mr Pole Evans writes: "When I visited Portuguese East Africa in September last, I saw some of the largest mango trees that I have ever seen at Quilimane and Inhambane. The trees were then in flower. I examined carefully for any disease that might be present, and saw no evidence of the bacterial disease. The inhabitants told me that the trees bore well and that the fruit was clean."

In the absence of any reference in available literature to any disease at all resembling the one under discussion, an attempt was made by various means to discover whether it occurred outside South Africa or not.

The symptoms accompanying the disease, which are very characteristic, were described to Dr Erwin F. Smith of Washington, and he stated that he was not aware of the existence of such a disease in America.

With reference to the possible occurrence of this trouble in India, Dr E. J. Butler, Director of the Imperial Agricultural Research Institute, Pusa, in a letter dated the 29th February, 1912, writes as follows:

"Last year for the first time a supposed bacterial disease of mango fruits was reported from the Bengal Agricultural College, Sabour, Bhagalpur. The Indian Assistant Professor of Mycology who examined the disease could find no fungus in the rotted spots on the fruit. Bacteria were naturally present, and he attributed the damage, which was considerable, to this cause. Some fruits were sent to my Laboratory and on examination I found the common *Gloeosporium Mangae* Noack (probably *G. Raciborski*, common on the leaves) together with a little of a Hendersonia which we have previously found associated with a mango fruit rot. I found no evidence to support the idea that the disease was bacterial."

At the suggestion of Mr R. A. Davis, we communicated with Mr Cousins, Director of Agriculture in Jamaica, who has devoted considerable time and study both to the cultivation of the mango and to the diseases to which it is subject. Mr Cousins wrote in reply:

"This disease is quite unknown in Jamaica. The mango sometimes suffers from the attack of thrips on the young foliage but otherwise is almost immune from disease and pests in Jamaica."

The majority of the trees in Natal were grown in the first instance from seed imported from Mauritius. Mr Bijoux, the Assistant Director of the Botanic Gardens, Mauritius, was visiting Durban in December, 1911. He stated that when he left the island the trees were perfectly healthy and laden with fruit. He knew nothing of the occurrence of such a disease. This information was obtained through the courtesy of Dr Medley Wood.

To summarise: so far as can be ascertained, there is no evidence of the occurrence of this disease outside South Africa. In all the mango-growing districts of the Union with the exception of the Zoutpansberg, the disease has caused considerable loss. Swaziland is also free from infection. In Portuguese East Africa cases of infection have only been reported from the neighbourhood of Delagoa Bay.

Symptoms of the disease.

Although the effects of the disease on the fruit are the most conspicuous, and of the greatest importance economically, numerous infections also occur on stems and leaves. The latter do not greatly affect the general health of the tree, but they serve to carry over the infection from one season to the next.

The first signs of infection noticeable on the leaves are a number of small, angular, water-soaked areas bounded by some of the veins of the leaf. These spots do not increase much in size and rarely exceed 2—3 mm. in diameter; but if they are very numerous they coalesce and larger spots are formed. The infected areas soon begin to discolour and become dark brown, the surface is somewhat raised and shining and frequently there is a slight exudation of gum (Plate II). In very old leaves these discoloured spots become white and dry, and crack away. If infection takes place in the petioles—as is often the case—longitudinal cracks result which attain a length of 1 cm. or less.

Diseased spots are also found on all parts of the stem, although it is evident that the majority of the infections take place in young and rapidly growing tissues; they are also common in the scars from which

leaves have newly fallen. The first appearance of disease in the stem is a discolouration of the tissues. This is accompanied by gummosis and the formation of deep longitudinal cracks. The discolouration is not merely superficial but penetrates some distance into the stem (Plate III).

The disease is especially evident on the peduncles and pedicels; frequently, by the time the fruit is half grown, the whole inflorescence is affected. The stalks to which the mangoes are attached become black and dead, and consequently the fruit all falls to the ground (Plate VI).

As has been mentioned above, although the spots on the leaves and stems are often very numerous, they do not noticeably affect the general health of the tree. Their chief importance is as a source of infection for the fruit which is seriously injured by the disease. Large numbers of mangoes fall to the ground, and the small percentage which remains on the trees is so disfigured as to be practically useless (Plate IV). The mangoes on the windward side of the tree suffer most. The diseased spots first appear on the most exposed side of the fruit or else at the spot where two mangoes on the same bunch are in contact and where a drop of water would lodge after rain. The first sign of infection is a small water-soaked area round the white spot which indicates the presence of a stoma, or near a slight wound; this spreads considerably and then begins to discolour. Cracking takes place in several directions and the surface of the diseased area becomes very much roughened. If infection takes place during a period of rapid growth, deep longitudinal cracks are formed, running almost the whole length of the fruit in bad cases (Plate V).

The discoloured spots vary much in size; they are 1 mm. to 15 mm. in diameter and are irregular in shape, and the discolouration penetrates to a depth of 8—15 mm.

Especially noticeable in the case of infections near the point of attachment of the fruit is the exudation of gum which runs over the surface of the mango. This substance is highly infectious and diseased spots are developed wherever it touches the surface of the fruit.

When a mango has once become diseased the slightest air movement detaches it from the tree, and the ground becomes strewn with decaying fruit.

Hosts and Varieties affected.

The disease has been observed on all the varieties of mango commonly grown in this country, but all are not equally susceptible.

The variety known locally as the Peach Mango or Figette is the most resistant. This is a very fine-looking mango and keeps well, but is said to be of inferior flavour.

The most susceptible is the "Baissac" variety, known locally as the "Long Green"; the other three varieties which are commonly grown, namely "Maison Rouge," "Corde" and "Dauphine," are almost as badly affected. These are known locally as the "Long Red," or "Red Kidney," the "Common Yellow" and the "Round Green," respectively.

In view of the fact that so far as is known at present this disease is confined to South Africa, search was made among nearly related indigenous plants for a source of infection. In particular, the "Maroola" tree (*Sclerocarya caffra*) was examined, as it is very common in districts where the mango is grown. The only specimen of this plant which showed any spots on the leaves proved to be infected with *Cercospora* sp.; and I have been unable to produce with the bacillus causing the mango disease, any infections on the leaves or fruit of the tree.

Spraying experiments.

A more detailed study of the progress of the disease was made during the season 1911—1912 in connection with spraying experiments which were being carried out at Barberton in an orchard kindly placed at our disposal by Messrs Winter Brothers. The season was an exceptionally dry one, and consequently unfavourable to the spread of the disease. The following table compiled from statistics furnished by the Meteorological Department shows that the rainfall has been much lower than in the previous seasons. The temperature has been exceptionally high.

Comparative Table. Rainfall 1907—1912.

	September		October		November		December		January		Total	
Season	No. of ins.	No. of days	No. of ins.	No. of days	No. of ins.	No. of days	No. of ins.	No. of days	No. of ins.	No. of days	No. of ins.	No. of days
1907–8	·14	2	2·18	9	4·14	15	4·15	10	3·62	9	14·59	45
1908–9	2·38	3	2·42	12	3·76	12	3·78	8	14·99	17	27·33	52
1909–10	·63	4	1·78	7	3·70	13	6·74	12	5·51	15	18·36	51
1910–11	1·14	5	4·13	14	3·88	13	5·41	13	7·95	10	22·51	55
1911–12	·07	1	4·63	13	2·35	10	2·61	12	1·74	9	11·40	45

The orchard in which the spraying experiments were carried out is situated on a slope to the east of the town, and contains 28 trees arranged in four rows of seven trees each. The trees are ten or twelve years old and comparatively small—about 12 feet high and 35 feet in circumference—so that the spraying could be thoroughly done.

There is a range of hills to the south of the orchard and the prevailing winds are from the south-east. The disease had started in the south-east corner of the orchard, and from there had spread right through. Before the experiments were commenced the trees were all diseased: row (1) being bad right through while in the other rows trees (6) and (7) were the worst.

Plan of Winter Bros. Orchard, Barberton.

East 200 ft.

						*	* ↙	
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	Row 1.
North	(1)	(2)	(3)	(4)	(5)	(6)	(7)	Row 2.
150 ft.	(1)	(2)	(3)	(4)	(5)	(6)	(7)	Row 3.
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	Row 4.

Four Rows of Mango Trees, 18 feet apart.
The arrow indicates the direction of the prevailing winds.
* These trees were the first to contract the disease.

Rows 1 and 3 were sprayed with an iron sulphide solution, using the following formula:

Quicklime	4 lbs.
Flowers of sulphur ..	4 ,,
Iron sulphate	1½ ,,
Water	25 gallons

and following the directions given in the circular published by this Division.

Rows 2 and 4 were sprayed with Bordeaux mixture, using a 2-2-50 formula for the first spraying and a 4-4-50 in subsequent applications.

In each row tree No. (3) was left unsprayed as a control.

The experiment was carried out by Mr P. A. van der Bijl, M.A., of this Division, and he was assisted in the earlier part of the work by Mr H. F. Benger. During the seasons 1912—1913 and 1913—1914, the spraying was also done by Mr van der Bijl, and it is from his reports that this account of the spraying experiments is compiled.

The following table gives the result of the experiment in connection with weather conditions, etc. The slight discrepancy between this and

the previous table is due to the fact that the first gives the rainfall in the whole district, the second in Barberton only.

Season 1911—12.

Week ending	Rainfall	Temperature Max.	Temperature Min.	Remarks on Weather	Treatment	Development of Fruit	Progress of Disease
26. 8. 11	None			No rain up to 30. 9. 11	1st spraying	Trees in flower	Old leaves badly diseased
7. 10. 11	2·66 in.	74°F.	52°F.	First heavy rain of season			
14. 10. 11	None						
21. 10. 11	·01 in.				2nd spraying	Fruit about the size of walnuts	No sign of disease on fruit
28. 10. 11	·61 in.	70°F.	58°F.	Three weeks since 1st rain		Fruit 1½″ to 2″ long	Diseased spots first noticed on fruit
4. 11. 12	·87 in.	74°F.	52°F.				
11. 11. 11	·08 in.	78°F.	54°F.		3rd spraying		About 5 % of fruit diseased
18. 11. 11	·46 in.	86°F.	54°F.				
25. 11. 11	1·29 in.						
2. 12. 11	2·06 in.	82°F.	64°F.		4th spraying	Fruit 3–4 ins. long	Disease spreading. About 7 % disease
9. 12. 11	·58 in.						
16. 12. 11	·45 in.						
23. 12. 11	·6 in.	98°F.	64°F.				
30. 12. 11	·5 in.	90°F.	58°F.				
6. 1. 12	·86 in.	100°F.	62°F.				
13. 1. 12		88°F.	54°F.				
20. 1. 12	·11 in.	92°F.	62°F.			Almost full grown	Fruit on all trees diseased 40–70 % affected

Diseased spots were first observed on the fruit about three weeks after the first rain of the season.

The final estimate of the percentage of diseased fruit refers only to the mangoes still on the trees; large numbers had fallen to the ground and rotted.

The result of the experiment was far from encouraging. On the 20th January an estimate of the number of mangoes still on the trees showed 30·7 % free from disease on the trees sprayed with iron sulphide

solution; 54 % on those sprayed with Bordeaux mixture and 67 % on the control trees.

A second spraying experiment was carried out during the season 1912—13, but unfortunately weather conditions were again unfavourable.

The spraying was done in the orchard described above, trees Nos. 3, 5 and 7 in each row being left as controls and the remainder being sprayed with Hycol in the proportion of half a pint to 40 gallons of water (1 in 600). The trees were sprayed four times, and at the time for the first and second sprayings the ground was disinfected with Hycol in the proportion of half a pint of Hycol to 20 gallons of water (1 in 300).

The season was an exceptionally dry one, the fruit developing slowly, and some trees failing to set any fruit at all.

Owing to the drought, the disease spread very slowly early in the season; the first infections on the fruit being observed early in December. The trees were sprayed for the fourth time on the 4th December and on this occasion it was computed that ·45 % of the fruit on the sprayed trees was infected and 1·2 % of that on the unsprayed.

In January the experiment was discontinued owing to the fact that the fruit had been severely cut up by a hailstorm on the 20th of December. On the 7th of January it was calculated that the unsprayed trees had dropped 36·1 % of their fruit and the sprayed trees 38·8 % since the previous examination. Of the fruit which remained on the tree there was 16·7 % of the fruit on the unsprayed trees diseased as compared with 7·3 % on the sprayed trees. The latter seemed to have made more growth than the former, and the Hycol had not damaged the fruit or foliage in any way. The trees which were sprayed with iron sulphide solution during the previous season had received a severe check.

The results of this experiment as far as they went were more encouraging than those of the previous one, but owing to their having been brought to such a premature end they were not sufficiently conclusive, and a further test was necessary. A table is appended showing the rainfall, temperature, etc., during the season.

The spraying with Hycol was continued during the season 1913—14. The first spraying was done on the 29th August, when the trees and the soil underneath them were thoroughly drenched with Hycol 1·600. The trees were then in flower.

Season 1912—13.

Week ending	Rainfall	Temperature Max.	Temperature Min.	Remarks on Weather	Treatment	Development of Fruit	Progress of Disease
11. 9. 12	Nil.	—	—	Sun and wind	Sprayed with Hycol	Blossoming	
18. 9. 12	,,	—	—	Hot sun		Blossoms and fruit developing slowly on account of drought	
25. 9. 12	,,	—	—	Sun and wind			
2. 10. 12	,,	83°F.	45°F.	Sun and cloud	Sprayed with Hycol		
9. 10. 12	,,	92°F.	45°F.	Sun and cloud			No new infections yet evident
16. 10. 12	,,	99°F.	50°F.	Sun		Very backward through drought	
23. 10. 12	Drops	107°F.	60°F.	Sun and cloud			
30. 10. 12	·49 in.	90°F.	52°F.	Sun and showers	Sprayed with Hycol		
6. 11. 12	Nil.	106°F.	62°F.	Sun			
13. 11. 12	,,	108°F.	59°F.	Sun and cloud		Fruit small through drought	
20. 11. 12	2·18 in.	97°F.	48°F.	Sun and rain			
27. 11. 12	Nil.	110°F.	55°F.	Sun			Disease now spreading rapidly
4. 12. 12	·05 in.	103°F.	66°F.	Sun and cloud	Sprayed with Hycol		
11. 12. 12	2·51 in.	105°F.	65°F.	Sun and storm		Much damaged by hail	Disease now spreading fast
18. 12. 12	·3 in.	95°F.	62°F.	Sun and cloud			
25. 12. 12	1·5 in.	96°F.	57°F.	Sun and heavy hail storm	Experiment discontinued	Nearly all fruit cut off by hail and the remainder bearing hail marks	

The second spraying was done with Hycol 1 : 300 on the 6th October, after the fruit had set and was 2—3 cm. in length. On the third application of the spray on the 15th of November, the fruit had attained a length of 4—5 cm. and the disease had begun to appear both on the sprayed trees and the controls.

On the 26th January, Mr van der Bijl paid his final visit to Barberton in connection with the spraying experiments. The fruit on both sprayed trees and controls had nearly all fallen, and most of those which remained were diseased. It is therefore evident that Hycol is useless in preventing the spread of the disease.

This was again a dry season. About $2\frac{1}{2}$ inches rain fell between the first and second sprayings, and about 2 inches accompanied by hail between the second and third. The fruit was slightly injured by the hailstones.

Mr Winter reported that the disease has this season spread through the valley, and that he has noticed diseased fruit on his farm at some distance from the village.

From the account of spraying experiments given above, it is evident that the sprays used up to the present are useless in combating this disease; and that iron sulphide is worse than useless as it injures the foliage and thus promotes the spread of infection. The mango being an evergreen tree it is impossible to check the disease by pruning as in the case of "fire blight" and other diseases of deciduous trees.

The risk of infection can be slightly reduced by gathering and burning all diseased fruit and leaves and by keeping the soil under trees damp with some germicide which will prevent the dust from blowing about in the wind and carrying with it the infective bacteria; but no remedial measures used up to the present can be recommended as being really effectual.

The cause of the disease.

A large number of diseased plants have been examined, and in every case the tissues of the discoloured areas in the leaves, stem and fruit were crowded with bacteria.

An organism was isolated from a diseased fruit from Barberton in November, 1909; as it occurred in the host it was a short rod with rounded ends about $1{\cdot}5 \times {\cdot}6\,\mu$. A pure culture was obtained without any difficulty. The same organism has since been isolated dozens of times from fruit and leaves of trees growing in Warmbaths, Barberton and several localities in Natal; in every instance an almost pure culture was obtained at once, except in the case of very old infections on the fruit where a number of yeasts and bacilli have established themselves.

The method used in isolating the organism was as follows: A fairly young infection on the fruit was selected, the surface seared with a hot knife, then a portion of the underlying tissues was cut out with a hot

sterile knife and dropped into a tube of sterile distilled water; from this a series of plates was poured.

A different method was adopted with the leaves because the organism diffuses very slowly into water from the leaf tissues. A leaf was washed in 1 : 1000 mercuric chloride, then in several lots of distilled water. A glass pestle and mortar similarly treated, and then an infected part of the leaf ground up in it with some sterile sand and a little distilled water. A drop of the liquid was put into a tube of medium and plates poured.

After the organism had been repeatedly isolated in the way described above, attempts were made to infect a number of young mango trees. The inoculation work has been rendered difficult by the entire absence of any greenhouse accommodation. The trees do not thrive in the open in Pretoria and though they have flowered for two seasons they have not developed any fruit.

It has already been shown that the majority of the natural infections take place in vigorously growing tissues during a spell of wet weather. There have been three very dry seasons since the inoculation work was commenced (1911—12), and it is almost impossible to obtain infections in the open in dry weather.

The few successful inoculations described below, were obtained during a short spell of rain and cloud. There have been a proportionally large number of failures, but in no case have any of the control plants become infected.

This phase of the work will be continued as soon as suitable greenhouse accommodation is available.

Direct infection experiments.

1. A small portion of the gummy substance exuding from an infected spot on a mango fruit was inoculated by needle pricks into the stem and petioles of a young mango tree. After about four weeks there were discoloured areas round the needle pricks, later longitudinal cracks were formed. The following season these stem and petiole infections were responsible for a number of spots in the leaves in their immediate vicinity. The controls remained healthy.

Cultures of the organism were obtained from the discoloured areas, whose tissues were crammed with bacteria.

2. Portions of diseased tissue were crushed, and the bacteria allowed to diffuse in sterile distilled water. Part of the liquid containing the bacteria was then allowed to stream over both surfaces of

three leaves of a young mango tree. Each leaf was covered for 24 hours with a large glass tube plugged with cotton wool. Characteristic dark angular spots appeared on the inoculated leaves at the end of four weeks.

There were no spots on the controls.

3. Leaves on a third tree were infected successfully in a similar way to that described in experiment No. 2, with the exception that the bacteria were allowed to diffuse in sterile beef bouillon instead of in distilled water.

The controls remained clean.

Inoculation with pure cultures.

4. A young agar streak culture was suspended in sterile distilled water, and the water was allowed to stream over both sides of two leaves of a young mango tree which were covered over for 24 hours as described in experiment 2. A period of cloudy and rainy weather succeeded the date of this inoculation and of those previously described.

Infections were visible on the 21st day, in the form of small, angular, water-soaked looking areas. After a few days these areas began to change colour and rapidly became black. The discoloured tissues were crowded with bacteria, and a pure culture of the specific organism was at once obtained.

There was no sign of infection on the controls.

5. A second experiment was conducted similar to the above, but small punctures were made in the leaf surface with a fine needle. In this case a large proportion of the infections occurred in the neighbourhood of the needle pricks.

Controls pricked with a sterile needle showed no sign of infection.

6. A set of detached mango fruits was washed in 1 : 1000 mercuric chloride, and then in distilled water. They were sprayed with a suspension of a pure culture of the organism and then covered over with a bell jar. After about three weeks there was a small discoloured area round some of the stomata; the blackened areas containing numbers of bacteria. The observation could not be carried further as the fruit was destroyed by *Gloeosporium* sp. No blackening was seen in the controls. Other attempts to infect detached leaves and fruit all resulted in failure, as, in spite of all precautions, they were always attacked by *Gloeosporium* sp. before any infections could show themselves.

7. During the season 1912—13 no inoculations were attempted, but the work was resumed in the spring of 1913. The weather was again

very hot and dry. A young culture was used, a number of young leaves being inoculated on the 13th October by means of needle pricks, and the tree covered over with a wet tent for 24 hours after inoculation. This resulted in a number of small infections chiefly in the region of the needle pricks.

8. A second young tree was inoculated on the 3rd of November. There were a number of very young leaves on the tree, which had not yet lost their red colour; in some of these minute punctures were made with a fine needle and the whole was sprayed with the suspension of a young culture in distilled water. The tree was covered with wet sacks for 48 hours after inoculation. After 27—28 days, there were a few infections on all the younger leaves of the tree, especially in the neighbourhood of the needle pricks and a pure culture of the specific organism was readily obtained from the infected areas.

In both the above experiments the controls were entirely free from disease.

9. Attempts were made in the season 1911—12 to infect some fruits of the "Maroola tree" (*Sclerocarya caffra*) but without success.

Natural methods of infection.

It has been noticed in every instance up to the present that the spread of infection is in the direction of the prevailing winds. It seems probable, therefore, that the wind is the principal agent in spreading the disease and that the organism is carried to a large extent with the dust from beneath the trees. It has been noticed that where the ground is kept free of decaying vegetation and soaked with some germicide, that the spread of infection is to some extent arrested. It has also been observed that when the trees are growing among long grass the infection does not spread nearly so rapidly as when the ground under the trees is bare. This supports the supposition that the infective bacteria are largely carried by the wind.

The younger leaves and the fruit are undoubtedly infected by the rain dripping from diseased leaves. An infected spot is almost invariably found where two fruits are in contact and where a drop of water can easily lodge.

I have noticed very few insects on the trees; they do not appear to feed to any great extent on the mango foliage, and one rarely sees any leaves disfigured by insects. The common stink-bug (*Anoplocnemis curvipes*) is sometimes found on the young foliage, but is probably not responsible to any great extent for spreading the disease.

Morbid anatomy.

Some difficulty was experienced in obtaining good sections, as the tissues, especially of the leaves, are very tough and are apt to tear out of the paraffin. The best results were obtained after fixing in acetic alcohol, which was used hot; and embedding in paraffin in M.P. 60°C. The stain which gave the best result was a combination of Ziehl's carbol fuchsin with light green.

The organism causes no hyperplasias and appears to be entirely confined to the parenchyma. It makes its way into the tissues through a slight abrasion of the surface; it seems very probable that it can also gain an entrance through the stomata, but I have not yet been able to obtain sections which would establish the fact. When once it has effected an entrance, the organism multiplies rapidly and invades the surrounding tissues (Plate VII). The bacteria are very plentiful in the intercellular spaces, and appear to wedge apart the cells and dissolve the middle lamella (Plate IX); one frequently notices in sections through the fruit, a cell which is apparently still untouched, but is completely isolated and surrounded by masses of bacteria.

In some cases the bacteria appear to be intracellular also, but it is almost impossible to judge whether this is really the case or whether the rods have been dragged over the surface of the cells in sectioning; probably the latter.

A good deal of gummosis takes place during the destruction of these cells, the walls of which become swollen and discoloured. The discolouration does not appear to be due to any staining caused by the bacteria but to the decomposition of the cells which are attacked.

The swelling of the disorganised cells is so marked that even in the leaf the surface of the affected area appears raised to the naked eye. The increased thickness of the diseased region is well shown in the section photographed in Plate X. In the fruit this is even more marked and so also is the exudation of gum from the diseased tissues.

The nuclei of the cells in the affected area are abnormally large, and stain deeply with the fuchsin; they are very conspicuous in sections through young infections on the fruit.

As the tissues become disintegrated and the cells killed, the bacteria disappear from the dead areas and are found in more deep-seated tissues (Plate VIII). They do not appear to be capable of attacking lignified tissues in a number of sections examined there was no trace of bacteria in the fibro-vascular bundles of fruit or leaf, although the

surrounding spaces were crowded with bacteria, numbers of which were lying in close contact with the lignified walls.

In the section represented in Plate VIII a number of yeasts were visible in the broken down cells of the surface tissues, but were not present in very great numbers.

For this reason, the infections in the stem very soon cease to increase in size, and can only take place near the growing tip. The diseased spots in the leaf are bounded by some of the smaller veins.

Morphology of the organism. Dimensions.

The organism is a short rod with rounded ends, varying considerably in size on different media.

In the host plant it measures from ·8 μ to 2·6 μ, by ·5 μ to ·7 μ. The majority are 1·5 to 1·8 μ by ·6 μ.

In a preparation made from a 24 hours old culture on nutrient agar (— 15 Fuller) and stained with aqueous Gentian Violet, the limits of length were ·9 μ and 2·7 μ; the breadth varied from ·5 to ·7 μ. The average was 1·5 μ × ·6 μ. The following are some actual measurements:

1·5μ × ·6μ	2·4μ × ·7μ
1·6μ × ·6μ	1·6μ × ·6μ
2·24μ × ·6μ*	2·3μ × ·6μ*
1·4μ × ·5μ	2·7μ × ·6μ*
1·4μ × ·6μ	·9μ × ·6μ

The rods marked * were just about to divide, and the last in the list was the result of a recent division.

In beef broth cultures 21—24 hours old, short rather thick forms predominate; the rods measure ·9 to 1·4 μ × ·6 to ·8 μ. The following are some actual measurements taken from a preparation stained with carbol fuchsin:

2·1μ × ·8μ	1·4μ × ·8μ
·9μ × ·8μ	1·7μ × ·6μ
·9μ × ·7μ	

In gelatine cultures the rods are exceptionally small, the majority measuring ·9 to 1 μ by ·5 to ·6 μ. In slides made from a gelatine plate culture three days' old, the longest observed were only 1·6 μ.

In solutions containing large percentages of sodium chloride, e.g., beef broth containing 7 to 8·75 % NaCl the organism grows out into long threads which are very variable in length and thickness (Plate XIII, fig. *a*). They are not septate, and a drop from a beef broth culture containing 7 % NaCl examined with the paraboloid condenser showed

several of these long filaments in fairly active motion. Some of the longest ones are swollen irregularly and might perhaps be classed as 'involution forms.' They are again mentioned later under this heading.

The following are some measurements of such filaments from a preparation made from a three days' old nutrient broth culture containing 8·5 % NaCl.

32μ × 9 μ – ·6μ	1·9μ × ·6μ
12·6μ × ·6μ	16μ × ·6μ
5·9μ × ·9μ	50μ × ·9μ

In cultures containing 7 % NaCl, the majority of the rods did not exceed 15μ in length, but in the higher percentages there were large numbers of very long filaments. .

Fission.

The method of fission was studied in an agar hanging block culture (1, p. 110). The smear on the block was made from a suspension in water of a four days' old culture in 2 % dextrose broth. During the observations the rods were exposed to a brilliant light from a Nernst microscope lamp. The temperature of the room was 25° C., but in the vicinity of the microscope varied from 27° to 30° C. owing to the heat from the lamp before mentioned. The lenses used were $\frac{1}{12}$ mm. Zeiss immersion and No. 12 compensating ocular; with these the changes in the shape and grouping of the organisms could be quite easily followed. Observations were made every five minutes and drawings with the camera lucida about every fifteen minutes.

At 11.15 a.m. two individuals were singled out for study which were lying close together and were apparently the result of the division of a single rod (Plate XII, fig. *a*).

For the first 1½ hours, there was very little change beyond a slight increase in the length of both rods. At 12.55 there was a slight constriction in the middle of one of them (fig. *b*); a transverse wall was formed and at 1.10 p.m. the division was complete (fig. *c*). The second rod increased to nearly three times its original length and then divided in a similar way (figs. *f*, *g*). After this, all the individuals under observation continued to divide rapidly; about 15 minutes elapsed from the first sign of constriction to the completion of division and in another 20 minutes the two new rods thus formed had attained their maximum size and had begun to divide again. The segments were not always of equal size.

Each rod as soon as it divided, pushed away and became completely separated from its neighbour. By a careful adjustment of the illumination, a delicate capsule could be detected surrounding each and preventing close contact.

The small colony which was being formed from the two rods originally selected was kept under observation until 4.30 p.m., when it consisted of 28 rods. At this time other colonies on the agar hanging block numbered 2—24 individuals. In most cases these were the result of the division of a single rod.

At 9 a.m. on the following morning the colonies were easily visible to the naked eye. Under the low magnification their structure was grumose.

Development in the agar hanging block was comparatively slow, doubtless owing to the fact that growth was anaerobic and that for part of the time took place under very strong illumination. After 24 hours the colonies appeared to the naked eye as small white pin spots, whereas a poured plate kept in the incubator at 30° C. for the same length of time developed surface colonies 5—7 mm. in diameter. The submerged colonies, however, were very little larger than those developed on the hanging block.

Grouping.

In young cultures on agar and gelatine, the rods are usually single, but in liquid cultures they frequently occur in pairs.

In the pellicle on the surface of beef broth cultures, chains are formed; these are composed of elements similar to the single rods and may comprise from 2—40 individuals. The chains are straight or curved (Plate XIV, figs. *a* and *b*) and very easily fall apart. There are no chains in the sediment which forms when the tube is in any way disturbed and the pellicle sinks to the bottom.

The chains are not disposed in any particular way, but the orientation is irregular.

Internal structure.

Young, actively dividing rods stain very evenly, but in the older cultures some changes of structure can be observed.

Rods from a ten days' old culture in 2 % dextrose broth when examined with the paraboloid condenser frequently show one or several highly refractive granules (Plate XIV, fig. *c*). In preparations made from very old agar cultures to test for the presence of spores, some of

the rods contained small bodies which stained red by Moller's spore stain.

In the sediment of old broth cultures, the rods stain very unevenly, and are apparently vacuolated. Numbers of them have a large central vacuole with a deeply staining portion at each end. In others, there are several bands of colour across the organism in addition to those at the poles.

Capsules.

It has already been mentioned that a delicate capsule could be detected round rods growing on beef broth agar. This was still more evident with dark ground illumination.

In the ring formed round the tube at the surface of liquid broth (1 × 15 F.) cultures, there are numerous organisms with a very definite capsule (Plate XIV, fig. *d*).

When stained by MacConkey's method, these capsuled bacilli take the stain much more deeply than the others; the capsule is unstained and shows as a colourless ring surrounded by the slimy mass in which it is embedded; the latter takes the stain and appears granular.

Spores.

Cultures of various ages and on numerous media were examined for spores but none were detected.

Motility.

In young cultures on agar and in liquid media, the organism is actively motile. The bacteria move rapidly with a forward screw-like movement which does not continue long in the same direction. The rod after proceeding for some distance turns and darts in a different direction, or its progress is interrupted by tumbling movements or by rotation on its long axis. The motile rods are usually single, but fairly frequently pairs may be seen in motion, or occasionally short chains. These move forward in a sinuous manner.

That flagella are present may be seen by using the paraboloid condenser with a Zeiss apochromatic objective 3 mm. ·95 ap. The number and position of the flagella cannot be ascertained, however, as they are in rapid motion, although it is evident that there are several and that they are peritrichous.

When observed under a cover glass, after a while the majority of the rods make their way to the edge of the preparation or to an air

bubble where they remain in feeble motion; the remainder gradually come to rest.

The flagella stain fairly easily by van Ermengen's method. They are three to eight in number, peritrichous, slender and 5—7 times the length of the rod (Plate XIV, fig. *e*). A more satisfactory and simple method of staining was found in one described in detail by Ellis (7). This is a modification of Löffler's flagella stain; it is a very simple method and gives a clear stain on a ground almost free from precipitate. I have failed to stain the flagella by the original method of Löffler, or by Pitfield's method.

Involution forms.

The long threads found in cultures containing large percentages of NaCl may possibly be termed involution forms. Some of these are curved and others are swollen irregularly (Plate XIII, fig. *a*). A drop of ± 15 beef broth was placed on a cover glass, inoculated with rods from a beef-broth culture containing 8·5 % NaCl (Plate XIII) and inverted over a moist chamber. The drops contained a couple of dozen rods of varying length and form; the rod shown to the right of fig. *a* was selected for observation.

In 30 minutes two septae had formed and ten minutes later the rod separated into three distinct lengths (fig. *c*). These two stages were observed with the $\frac{1}{12}$th oil immersion (Zeiss) and No. 12 compensating ocular and were drawn with the camera lucida. At this point, however, the rods moved into a deeper part of the drop and it was not possible to get them into focus with the oil immersion. The observation was therefore continued with the Zeiss objective D and compensation ocular No. 18. The drawings were done freehand as near as possible to the scale of the first two.

The portions of the original filament now divided fairly rapidly into segments of very unequal length, and after three hours they became approximately the normal size of the bacillus (figs. *d—g*). At this time, the rods being small and rather numerous, they scattered, and it was impossible to trace them further as they became intermingled with bacilli resulting from the division of other filaments.

Some of the rods divided into much more equal segments than the one selected for observation, and the segments remained in contact longer. Fig. *h* shows an exceptionally long one and two shorter ones which had divided in this way.

The long filaments are not rigid and changes of form are frequently observable through curving in various directions.

The culture was exposed to the light of a Nernst lamp during the period of observation and the temperature of the room was 26—27° C.

The slide was placed in the incubator at 30° C. during the night and was again examined the following morning, 22 hours after the culture had been made. The drop was crowded with rods of normal size; in the centre was a thick clump, cochleate in form, but all the outlying rods were in active motion.

Staining reactions.

The organism stains well with all the ordinary aniline dyes; it stains deeply with dilute aqueous solutions of methylene blue, basic fuchsin, thionine and gentian violet; and still more intensely with carbol fuchsin and carbol gentian violet. The last named seems to be the best stain for general purposes.

The bacillus is not acid fast, i.e. it stains blue by the Ziehl-Neelson method but is Gram-positive, and also stains by Claudius' method which has recently been recommended for use in place of Gram's method (2).

When stained by Neisser's method, the bacilli are light brown, and many of them show a small black granule at each pole which is stained with the acid methylene blue. The rods which showed this reaction had been grown on Löffler's blood serum and incubated at 37°C. for 18 hours.

Cultural characters.

In all cultures made for the observation of morphological and cultural characters, preliminary cultivation was practised as prescribed in the chart issued by the Society of American Bacteriologists (1), except that as growth was very slow at 20°C. the cultures were incubated at 25°C. In describing the topography of the colonies, terms are used as defined by Chester (4) and the references following names of colours are to the numbers of the plates in which the corresponding colours can be found in Ridgway's *Colour Standards and Nomenclature* (12).

Nutrient Agar Colonies. Nutrient agar (+ 15 Fuller) was found to be a suitable medium for general purposes. The most characteristic colonies are developed at a temperature of 25°C. They are visible after 24 hours, when they are shining white, circular bodies 1 to 1·5 mm. in diameter; even at this early stage the margin of some of them is becoming undulate; they are denser at the centre than towards the

circumference and, when examined microscopically, are granular in texture. The submerged colonies are minute, irregular in form and granular (Plate XI, fig. *a*).

After 48 hours the colonies are 1—2 cm. in diameter, rather irregular in form and with a lobate margin. There is a dense spot in the centre surrounded by one or two concentric rings. The colour is white by reflected light, and a coppery tint by transmitted light. The colonies do not increase in size after this, but change somewhat in colour and texture up to the fifth day; at this time the colour is maize yellow or buff yellow (IV, *VO—Y, f—d*) and the colony is surrounded by rather a heavy margin (Plate XI, fig. *a*); the surface is smooth and shining, and slightly raised.

In thickly sown plates the colonies are irregularly circular, with a less deeply lobed or almost smooth margin.

At 30°C. the form of the colonies is somewhat different; they attain a diameter of 1—5 mm. in 24 hours. They are opalescent by transmitted light, with some concentric rings, finally becoming yellow as at 25°C. The maximum diameter is 6—10 mm. and the colonies are irregularly circular with a heavy margin.

At 37°C. growth is also rapid, the colonies are more opalescent than at 30°C. and the concentric rings are more conspicuous. When mature, the colonies are circular and the margin smooth; the colour is slightly deeper than at the lower temperature. The centre of the colony is very slightly raised, and in some there is a heavier ring about 1—2 mm. from the edge, with radiations towards the margin.

The growth at 20°C. is comparatively slow. Colonies are only just visible to the naked eye after 24 hours when they consist of a dense white spot surrounded by a translucent margin. At this temperature thin, spreading colonies are finally formed 7—8 mm. in diameter. They are irregular in shape, and no definite yellow colour is developed; the colonies remaining a creamy tint (19 *yo—y*).

In old plate cultures, especially those at the higher temperatures, there are numerous X-shaped crystals formed (Plate XI, fig. *b*). In all young cultures when examined microscopically, a swaying movement is noticeable right through the colony, due to the activity of the rods composing it.

Nutrient agar streak. On nutrient agar with a reaction of + 15 Fuller, there is quite a plentiful growth after 24 hours at 30°C. It takes the form of a glistening homogeneous streak along the needle track which is somewhat opalescent by transmitted light, milky white by reflected light.

After 48 hours streaks average ½ cm. in width, but taper gradually from a breadth of about ¾ cm. at the bottom of the tube to a point near the top of the medium. The difference in the luxuriance of the growth at different levels is no doubt due to difference in the amount of moisture in the medium. The edge is smooth and there is a homogeneous translucent margin about 1·5 mm. wide all round the growth, the central part has become more dense and granular. In some of the tubes there are small opaque spots in this central portion or else longitudinal lines of a similar nature.

The streaks do not usually exceed 1 cm. in width when growth ceases, but if the surface of the agar is very moist, sometimes a spreading growth is formed which almost covers the surface of the agar. Occasionally there is a tendency to form discrete colonies.

The yellow colour is visible after four days, being similar to that developed in the plates. The edge of the growth also becomes heavier than the central portion. The surface is always shiny, and when the medium is becoming rather dry it looks almost like varnish. A heavy yellow sediment forms at the bottom of the condensation water, and on the surface of it there is a pellicle which adheres to the glass tube on the side remote from the slant agar.

The colour of streak cultures is often a little deeper than that of colonies, and varies from buff-yellow to apricot yellow (IV, 19 *YO—Y d—b*). In old cultures X and Y-shaped crystals are formed starting from the surface of the medium and pointing down into the agar. The surface of the agar becomes whitish. There is no noticeable odour.

Nutrient agar stab. There is very little growth in the depth of the medium, only a thin white line following the needle track. A fairly large round colony is formed on the surface of the agar.

Glucose formate agar. A number of cultures were made on this medium as controls to anaerobic cultures. The growth is similar to that on nutrient agar, but if anything more luxuriant and slightly deeper in colour. Crystals are very frequently formed in this medium.

Mango agar. The growth on agar made from an extract of mango fruit was somewhat similar to that on beef-broth agar, but the culture is cream coloured and never becomes yellow. It consists of a glistening streak along the needle track, with smooth edges and very much raised surface.

Treacle agar. The organism only made a very feeble growth on this medium.

Sol. N. with agar. On Marshall Ward's Solution N. solidified with agar the organism grew fairly well for the first 24 hours, but did not subsequently extend very much. There was no chromogenesis; the streak did not exceed 4 mm. in breadth, and was granular round the edges.

The bacillus grew out into very long threads on this medium, but the filaments did not show the irregularities characteristic of those grown in NaCl solutions.

Nutrient gelatine (+ 15 *Fuller*) *colonies.* When incubated at 20° C., colonies on nutrient gelatine are just visible to the naked eye after 24 hours. After 48 hours the superficial circulars are $\frac{1}{2}$ mm. to $\frac{3}{4}$ mm. in diameter. They are circular, white and glistening, and the surface being very much raised they have the appearance of small drops of milky water. The submerged colonies are smaller and irregular in outline. All are granular as seen with the microscope under a low magnification.

In three days the surface colonies have increased still further in size; they are circular to ellipsoid, and measure up to 1 mm. The smaller colonies are capitate, the larger ones raised; the margin is wavy and texture coarsely granular; a yellowish tinge is becoming evident, and there is just an indication of liquefaction round the edge of the colonies. The submerged colonies are punctiform to the naked eye; when examined under magnification the majority are spherical, though a few are irregular in outline; they are granular in texture, dense in the centre and thinning out to a pellucid margin.

The surface colonies can be lifted out entire on the point of a needle. When mounted in water and examined microscopically, the bacteria diffuse very slowly from the compact mass, but those which escape into the water are very active.

After seven days, the surface colonies measure 1—3 mm., and submerged colonies $\frac{1}{4}$—$\frac{3}{4}$ mm. in diameter. The former are very much raised, somewhat moruloid, yellow and with irregular margin; the shape is also irregular.

After ten days, each of the surface colonies is sunk in a little saucer of liquefaction, and after 17 days the gelatine is completely liquefied. The yellow colonies are still entire and floating in the liquid medium, though larger and looser in texture than formerly. The colour is similar to, but slightly deeper than, that developed in the agar colonies.

At 12—15° C. similar results were obtained, but growth was decidedly slower. Colonies were not visible to the naked eye until after 48 hours;

the first distinct evidence of liquefaction (colonies crateriform), was noticed after 14 days, and liquefaction was complete in about 24 days.

Nutrient gelatine stab. A number of stab cultures, made in + 15 nutrient gelatine in test tubes measuring 7 × 1″ and incubated at 20° C., showed the following characters.

After 48 hours at 20°C., the needle track was just distinguishable; a thin line of white growth extending to the bottom of the tube. Three days later the growth in the deep parts of the gelatine was seen to consist of very numerous and minute spherical colonies. On the surface of the gelatine there was a colony about 4 mm. in diameter and rather deep coloured.

In nine days the surface growth had sunk in a little saucer of liquefaction, and under this there was a hemisphere of liquefied, clouded gelatine. Five days later the top of the liquefied portion was 1 cm. in diameter; the surface colony remained entire, floating on the liquefied gelatine. Its centre was deep coloured, but it was lighter towards the margin. The shape of the whole growth was napiform, 1·5 cm. wide at the broadest point. There was a sediment at the bottom of the liquefied portion.

In twenty days from the time of inoculation the growth had extended to the width of the tubes and the liquefaction to a depth of 2 cm. (Plate XI, fig. *c*). The cloudy, liquefied portion continued to increase in size, became infundibuliform in shape (Plate XI, fig. *d*), then extended downwards and finally completely liquefied the gelatine.

The colour of the surface growth was deep chrome (Plate III, 17 *O—Y*, *b*) and that of the sediment light orange yellow. The culture had no distinctive odour.

In tubes inoculated at 25°C. liquefaction commenced after six days and reached the stage shown in fig. *d* in nine days.

Nutrient gelatine streak. Streak cultures on nutrient gelatine incubated at 20° C. form a line of growth about 5 mm. broad along needle track in three days. It is smooth, shining and yellow, and opalescent at the edges. In a short time liquefaction commences at the bottom of the streak, making a groove in the gelatine, and as it continues the melted gelatine runs to the bottom of the tube.

Nutrient gelatine shake. Shake cultures in nutrient gelatine developed very numerous, minute colonies near the surface of the gelatine becoming less numerous towards the bottom of the tube; they are evident after three days. Liquefaction sets in on the sixth day, beginning at the surface of the medium and working downwards until the whole is liquefied.

Löffler's blood serum. Streak cultures on Löffler's blood serum at 37°C. at the end of 18 hours show a shining, yellowish growth along the needle track; after 48 hours this becomes deep chrome yellow, forming a streak about 2 mm. broad with smooth edges; it is shining and not raised above the surface of the medium. No liquefaction is observed in tubes kept under observation for several weeks.

Starch Jelly. There was no growth in starch jelly made with Uschinsky's solution even in tubes kept under observation for several weeks.

Potato. The first evidence of growth on potato cylinders is a slight, shining yellowish growth along the needle track; it then spreads over the lower, moist portion of the cylinder, but on the upper half there is no further growth. The colour is buff yellow (IV). There is no greying or other discolouration of the medium.

Mango. On pieces of mango sterilised by steaming in Roux tubes the organism forms a cream-coloured, spreading growth covering the surface of the medium. The mango was not discoloured.

Cocoanut. This is a good medium for chromogenesis, but a plentiful growth is not obtained unless the medium is moist. At 25°C. and 30°C. growth was just visible in 24 hours, first appearing as a shining streak along the needle track; in 48 hours a glistening, cream-coloured growth covered all the lower part of the cylinder. This was the wettest part of the medium, as the pieces of cocoanut were steamed in Roux tubes whose bulb was filled with water, or in ordinary test tubes resting on a wad of wet cotton wool. In three days the colour was slightly deepened, the water in the bulb of the Roux tubes clouded, and in tubes where the cocoanut was resting on cotton wool, the upper layers of this substratum were slightly yellow. The colour deepened and became buff yellow (IV. 19 *YOI—Y, d*). In the Roux tubes, a yellow sediment formed at the bottom of the bulb, and a ring on the glass just above the liquid. As soon as the medium began to dry up growth ceased, and no further changes took place in the appearance of the culture.

Beet. Tubes containing cylinders of beetroot were prepared in a similar way to those containing cocoanut. The organism grows very vigorously on beet; the whole of the cylinder became covered with a shining, wet-looking growth, the upper part having a granular appearance, and the lower portion being wrinkled. In the Roux tubes a heavy pellicle was formed on the surface of the water in the bulb, and in the straight tubes where the cylinder was resting on wet cotton wool

the surface of the latter was covered with a yellow wrinkled growth. The colour was deeper than on most media, varying from light orange yellow to deep chrome (III 17, *O—Y*, *d—b*).

Carrot. This was not a favourable medium, a thin spreading growth covered the moist parts of the cylinder.

Nutrient broth (+ 15 Fuller). The bacillus grows very vigorously in nutrient broth; at 30° C. a slight clouding is visible in 6—8 hours; after 24 hours a ring begins to form above the surface of the liquid, and in 48 hours it is well formed and yellowish in colour. A thin pellicle forms on the surface of the broth, and this sinks to the bottom if the tube is slightly shaken and forms a sediment. After three months the broth becomes almost clear, the ring has dried on the sides of the tube, and all of the pellicle fallen to the bottom.

The ring formed above the surface of the broth is very tough and slimy; it is almost impossible to break it with a platinum needle. Cultures in nutrient broth had no noticeable odour.

In broth containing 2 % dextrose, similar results are obtained, but the clouding is heavier; in a medium containing 6 % glycerine, on the other hand, there is a much less copious growth.

Dunham's solution. The bacillus does not grow well in peptone water, but this medium becomes slightly clouded in 48 hours. There is no pellicle and no sediment.

Litmus milk. The tubes when inoculated were deep lavender in colour (XXXVI) and the controls did not change during the time that they were kept under observation.

The organism grows very slowly in milk tubes, and it was not until the tenth day that there was any decided change. After 12 days at 25° C. the colour was pale lilac (XXXVII); on the 16th day it was flesh pink (XIII), and this change of colour was accompanied by a curd-like coagulation of the casein with a separation of whey.

At 30° C. a similar coagulation took place after the same lapse of time, but the reaction was not so acid, the colour being pale cinnamon pink (XXIX). At these two temperatures no further change of colour took place, nor was there any solution of the curd; but after 25 days the colour was completely reduced in all the tubes.

At 37° C. the medium did not turn pink, but after 12 days the colour was completely reduced. The casein coagulated but there was no extrusion of whey. The curd was then gradually dissolved, and after 27 days all that remained was a clear yellow fluid with a slight bacterial sediment at the bottom of the tube.

Uschinsky's solution. The bacillus would not grow in this solution.

Cohn's solution. A slight clouding took place after three days. No ring or pellicle formed, and there was no sediment.

Beerwort. The tubes were kept under observation for a considerable length of time, but they remained clear.

Potato broth. Abundant growth took place in Appel's potato broth. A ring and pellicle were formed.

Cabbage broth. In cabbage broth the organism caused very heavy clouding; there was not much pellicle, but a fairly wide ring formed, and there was about $\frac{1}{2}$ cm. of sediment at the bottom of the tubes. The upper part of the ring was yellow, but the lower part, which was in contact with the broth, was light salmon orange (11). The sediment was the same peculiar colour.

Beet juice. Very heavy clouding took place in this medium, the ring and pellicle were very well developed in colour, the growth was similar to that formed on solid beet.

Solution N. A fair amount of growth was observed in Marshall Ward's Solution N, the medium clouded, but there was no formation of ring or pellicle.

Physical and Biochemical Features.

In this section of the work, unless otherwise stated, the methods used are those outlined by Eyre (8). Each of the tests was repeated several times, and in no case were the results contradictory, although they varied slightly with the vigour of the culture used. Even where not specially mentioned, each experiment was checked by the use of controls.

Enzyme production. The organism is capable of dissolving the middle lamella of cells in the tissues of the host plant, but it has apparently no action on cellulose.

A series of experiments conducted to test for the presence of various enzymes in cultures of the bacillus, for the most part led to negative results. A brief outline of these experiments follows.

Diastatic enzymes. Four tubes of nutrient broth of optimum reaction were inoculated and incubated for five days at 30° C. The cultures were removed from the incubator on the fifth day, and mixed with equal quantities of starch paste containing 2 % of thymol; they were then placed in the incubator at 37° C. for six hours, and at the end of that time were tested for sugar with Fehling's solution. There was no reaction for sugar.

Cylinders of potato on which the organism had been growing for ten days or longer, when tested with iodine gave the purple red reaction characteristic of amylodextrin, but did not reduce Fehling's solution.

Invertin enzymes. Tube cultures in nutrient broth were prepared and incubated as in the previous experiment. At the end of the incubation period the cultures were mixed with an equal quantity of a 2 % solution of saccharose containing 2 % phenol and allowed to stand for six hours. When tested at the end of this time, it was found that the mixture did not reduce Fehling's solution, therefore there were no invertin enzymes present.

Rennet and lab enzymes. Tubes prepared and incubated as before were heated at 55° C. for 30 minutes, in order to sterilise them without destroying any enzymes which might be present. After this 5 cc. of the culture was run into each of three tubes of sterile litmus milk. No coagulation or change of any kind was observed in these during ten days at 20° C.

Acid production. There is no very marked change in the reaction of any liquid medium in which this bacillus is grown. A series of flasks containing nutrient broth with 2 % lactose, saccharose, glycerine, laevulose and dextrose were inoculated and kept at a temperature of 30° C. for ten days; at the end of that time the cultures were slightly more acid than the controls, but only by 2—5 degrees of Fuller's scale.

A test for organic acids was also made, using 500 cc. of 2 % dextrose broth in which the organism had been growing for ten days. The only reaction obtained was a rather doubtful one for succinic acid.

Alcohol production. The first distillate of a ten days old culture in 2 % dextrose broth was divided into three portions.

To the first was added Lugol's iodine solution, then a little NaOH solution. There resulted a smell of iodoform indicating the presence of alcohol, acetone or an aldehyde. A small quantity of Schiff's reagent was pipetted into the second portion; in the greater number of cultures tested there was no reaction, but in one case a faint pink colour appeared, and there was probably a trace of aldehyde in the culture.

To 10 cc. of the third portion was added 10 cc. of H_2SO_4 and 1 cc. of a 4 % solution of potassium permanganate. On adding Schiff's reagent, after five minutes a decided red colour was developed, indicating the presence of alcohol. A decided reaction for alcohol was obtained repeatedly in the distillate from cultures in 2 % dextrose broth.

Ammonia. Two flasks of nutrient broth were prepared, each containing 100 cc. One of these was inoculated, and both were put in the

incubator at 30° C. After ten days, 2 grams of calcined magnesia were added to each flask, and 50 cc. distilled from each. Neither of the distillates gave any reaction for ammonia with Nessler's solution. Ammonia is sometimes produced in media containing a nitrate, but this point will be discussed under the heading "nitrate reduction."

Indol and phenol. Tubes of peptone water in which the organism had been growing for ten days at 30° C. always gave a distinct reaction for indol with sulphuric acid and potassium nitrate, there was no such reaction in the controls.

A culture in a flask containing 300 cc. of nutrient broth was used to determine the presence or absence of phenol. After ten days at 30° C. the contents of the flask were tested for indol and phenol as follows. After adding 50 cc. of HCl, the flask was connected with a condenser and 50 cc. distilled over. The distillate was rendered strongly alkaline with KOH and redistilled. The distillate tested with sulphuric acid and potassium nitrite gave a decided reaction for indol.

The residue, when cold, was saturated with CO_2 and redistilled. This third distillate gave no reaction for phenol with Millon's reagent or with ferric chloride. A control flask, similarly tested, gave no reaction either for indol or for phenol.

Pigment production. It has been stated in connection with the description of the cultural characters of the organism that it is capable of producing a yellow pigment on a variety of media; and that this pigment develops more rapidly at 30°—37° C. than at lower temperatures. The colouring matter is insoluble in water, hot or cold, in alcohol, ether, chloroform or dilute acids.

Colour reduction. The organism was grown in nutrient broth tinted with various coloured substances; a number of trials were made in each, but a typical set of tubes is selected in each case for detailed description. In all the experiments the colour of the control tubes remained unchanged.

Litmus. The reduction of litmus in milk cultures has already been described. The colour is also reduced in nutrient broth with or without the addition of dextrose. In a vigorous culture reduction is complete in 48 hours at 30° C., but with a less vigorous strain of the bacillus the process was much slower and took as long as six days.

In one instance the tubes before inoculation were bishop's purple (XXXVII), in 24 hours the reaction of the tubes was more acid, and the colour scarlet red (I). The following day reduction had commenced, working from the bottom of the tube; the lower half of the broth was

light salmon orange (II), and the upper half peach red (1). After three days the upper third of the tube only retained any colour and in five days it was colourless. The only considerable variation in this process was in the length of time which it occupied; the medium in every case became slightly acid and then bleached out from the bottom upwards.

Neutral red. Tubes of nutrient broth tinted carmine (I) with this stain were inoculated and incubated at 30° C. The organism grew well in this medium, and a fairly heavy ring formed round the tube at the surface of the liquid. The cultures were kept under observation for ten days, but no reduction took place. The bacterial ring absorbed the colouring matter and became carmine. Rods from this ring when mounted in water and examined microscopically were faintly stained; there were some short capsuled rods, and some long filaments of rather uneven breadth similar to those found in media containing NaCl.

Rosolic acid. The colour of the medium at the beginning of the experiment was peach red (I). After 24 hours at 30° C. the colour was bittersweet orange (II), and it subsequently passed through buff (III) to light orange yellow (II). In six days the culture was entirely colourless except for the bacterial ring at the surface of the liquid which had absorbed the colouring matter and become orange red. The stain was not in this case sufficiently intense to be evident in the bacilli when they were examined microscopically.

Methylene blue. A number of tubes and fermentation tubes were prepared containing broth tinted with methylene blue to the colour of a strong ammoniacal solution of copper sulphate. The closed arm of the fermentation tube was colourless after sterilisation and remained so throughout the experiment; the description of changes of colour therefore refers to the ordinary test tubes, and to the open arm of the fermentation tubes.

As in the case of tubes coloured with litmus solution reduction commenced at the bottom of the tubes. After the cultures had been kept at 30° C. for 24 hours the lower part of the tube was colourless and the upper part Tyrolite green (VII). On the second day there was only about $\frac{1}{2}$ cm. at the surface of the liquid which retained the green colour. After six days the medium was colourless, but the bacterial ring had partially absorbed the colour and was Venice green (VII).

The colour did not return at all when the tubes were shaken.

Indigo carmine. Indigo carmine was also gradually reduced from the bottom of the tube upwards. In this case the organism did not absorb the colouring matter. Reduction was complete in eight days.

Congo red. Congo red was not reduced, but the dye was absorbed by the superficial bacterial growth as in the case of neutral red.

Cultures were made in nutrient broth tinted with several other dyes, chiefly in order to determine whether any of these would inhibit the growth of the organism. None of them actually inhibited the growth in very dilute solutions, but growth was feeble in broth containing malachite green or cyanin.

The bacillus grew well in Trypan blue, and completely reduced the colouring matter; Trypan red was not reduced, but the surface growth absorbed the stain.

Reduction of nitrates. Nitrates were more completely reduced when the organism was grown in nitrate water than when it was grown in nitrate broth.

A flask of nitrate water (8, p. 169) was inoculated from a young culture of the organism in nutrient broth and incubated with a control flask at 30° C. for five days. The culture was then divided into four portions, the first two being used to test for the presence of nitrites. There was no reaction for nitrites when tested by the iodine-starch method or with α-naphthylamine and sulphanilic acid.

A third portion tested with Nessler's solution developed a distinct yellow colour indicating the presence of ammonia. The remainder of the culture was evaporated to dryness and tested for the presence of nitrates with phenolsulphonic acid. There was no reaction.

The nitrate water in the control flask tested in a similar way gave a distinct reaction for nitrates but none for nitrites or ammonia.

The test for nitrate reduction was repeated using nitrate broth (8, p. 143) in place of nitrate water. The culture and control were tested on the fifth day in the manner described above and as before the control was found to contain nitrates but no nitrites or ammonia. The culture reacted strongly both with iodine and starch paste, and with α-naphthylamine and sulphanilic acid showing the presence of nitrites; there was no reaction, however, for nitrates or ammonia.

Fermentation tubes. The organism was grown in fermentation tubes containing 2 % sugar broth, but there was no gas production with any of the carbohydrates used. In one solitary case gas was formed from dextrose and saccharose, but this proved to be due to the presence of an interloper. The vigour of growth in the open and the closed end varied greatly with the composition of the medium. The intensity of clouding

in media containing various carbohydrates is shown in the following table:

Carbohydrate used	Clouding in closed arm	Clouding in open arm
Dextrose	heavy	very heavy
Saccharose	heavy	very heavy
Laevulose	moderate	heavy
Maltose	moderate	heavy
Raffinose	slight	heavy
Galactose	very slight	moderate
Mannite	slight	heavy
Lactose	none	fairly heavy
Dextrin	very slight	very heavy
Glycerine	none	moderate

It will be noticed from this summary, that the organism does not grow anaerobically in the presence of glycerine and lactose, and only feebly in the presence of galactose and dextrin.

Hydrogen sulphide. Tubes of peptone lead solution (8, p. 186) were prepared in order to test for the presence of H_2S. Cultures in this medium were kept under observation for sixteen days, but there was no formation of hydrogen sulphide; the precipitate was not blackened. At the end of sixteen days the cultures were discarded.

Toleration of NaCl. The bacillus can grow in media containing comparatively large percentages of sodium chloride. A number of tubes containing various percentages of NaCl (from ·5 % to 10 %) in + 15 nutrient broth were inoculated from a young culture and incubated at 30° C. Tubes containing less than 4 % NaCl were clouded in 18 hours, while those containing from 4 % to 8·25 % were clouded in 48 hours. In tubes containing 9 % and over there was no growth; in the 8·5 % and the 8·75 % solution the clouding was so slight as to be barely noticeable, but a microscopic examination showed that the organism had multiplied considerably. The abnormal forms found in this medium have been described elsewhere.

Reaction of medium. The organism is not particularly sensitive to the reaction of the medium in which it is grown. The optimum is about + 17 of Fuller's scale, but there is very little variation in the rapidity of growth in media of reactions varying from +14 to + 23 Fuller. During an experiment undertaken to find the optimum reaction, broth tubes having these reactions clouded in six hours at 30° C.; those with a reaction of + 2 to + 14 and from + 24 to + 27 Fuller clouded in seven hours; those with reactions from + 28 to + 30 and from + 10 to + 1 in eight hours. In tubes containing more alkaline broth growth

was considerably slower, the organism preferring a medium which is slightly acid to phenol-phthalein.

A series of cultures was made to test the ability of the bacillus to grow in media containing varying quantities of certain acids and alkalis.

To flasks containing 50 cc. of neutral broth were added different percentages of acetic, oxalic, tartaric, and malic acids, and of sodium hydrate and sodium carbonate. With each of these substances a series of flasks was prepared varying in reaction from 0 to ± 50 of Fuller's scale, the intervals between the reactions of any two in a series being five degrees, so that there were ten flasks in each series. The contents of each flask were pipetted into four tubes and these were sterilised. A loopful of a young broth culture was introduced into three tubes of each set and the fourth kept as a control. The results obtained from the observation of these cultures may be tabulated as follows, the controls in every case remained clear.

Substance tested	Amount to retard growth Reactions in terms of Fuller's scale	Amount to inhibit growth
Acetic acid	+ 25	+ 30
Oxalic acid	+ 30	+ 35
Tartaric acid	+ 35	+ 40
Citric acid	+ 30	+ 40
Malic acid	+ 35	+ 35
Sodium hydrate	− 30	− 45
Sodium carbonate	− 30	− 35

Atmosphere. That the organism is a facultative anaerobe was suggested by the fact that it grew in the closed end of fermentation tubes and in the depth of the medium in stab cultures, though in the latter growth is never abundant. A series of cultures was therefore made to test the ability of the bacillus to grow in the absence of oxygen and in the presence of various other gases. Bulloch's apparatus was used in making most of the anaerobic cultures, and the method used in each case was similar to the one now to be described; slight variations in method were of course necessary in order to introduce the various gases into the apparatus, but these are mentioned in connection with the individual experiments.

Oxygen and carbon dioxide absorbed. Streaks were made on slant nutrient agar and glucose formate agar, and a loopful of a young broth culture introduced into tubes of nutrient broth and glucose formate broth. These were placed in a beaker standing in a glass basin at one

side of which was placed a small heap of dry pyrogallic acid. The whole was next put inside the Bulloch's apparatus, the bell jar carefully sealed on to the ground glass plate and the air partially exhausted from the apparatus by attaching the exit tube to a suction pump. A strong solution of KOH was then siphoned into the glass basin without admitting air, dissolving the pyrogallic acid. A strong alkaline solution of pyrogallic acid was thus formed which absorbed the oxygen and carbon dioxide within the bell jar.

The apparatus was kept at a temperature of 30° C. with a number of control tubes. In 48 hours there was a very vigorous growth in all the control tubes; the growth in the tubes deprived of oxygen was exceedingly slow as compared with that in the controls, and was not at all abundant.

This experiment was repeated a number of times with similar results.

Hydrogen. A set of cultures was prepared and placed in the Bulloch's apparatus as described in the previous experiment. In this case, however, hydrogen was generated, and after being purified by passing through wash bottles containing solutions of silver nitrate, potassium permanganate and potassium hydrate, it was allowed to stream through the apparatus until all the air was expelled. Any oxygen or carbon dioxide remaining in the bell jar was absorbed as before with an alkaline solution of pyrogallic acid.

The growth in the tubes in the atmosphere of hydrogen was slightly better than in those kept in an atmosphere composed chiefly of nitrogen. This was found to be the case in two successive experiments.

A set of tubes inoculated from hydrogen cultures and grown in Buchner's tubes in the absence of oxygen made better growth than the cultures deprived of oxygen which had been made direct from aerobic cultures.

Carbon dioxide. The apparatus was prepared in the same way as before and a current of carbon dioxide passed through it. In this case any remaining oxygen was absorbed by means of a solution of pyrogallic acid in water. There was a slight growth in all the tubes at the end of 48 hours, but not quite so much as in those grown in an atmosphere of hydrogen. Growth was abundant in all the control tubes.

Sulphur dioxide. In this case SO_2 was passed through the apparatus until all the air was displaced before the introduction of the KOH solution. There was no growth in any of the tubes, nor did any growth

take place when the tubes were removed from the apparatus and placed in the incubator, the organism having been killed by prolonged exposure to the gas.

The ability of the bacillus to withstand short exposures of SO_2 was also tested. Transfers were made from a young culture to ten tubes of slant agar, a generous quantity of the culture being used. Two were placed in the incubator as controls, and the remainder were exposed to a stream of SO_2 for periods varying from 15 seconds to five minutes. In tubes exposed for 15 or 30 minutes there was a slight growth along the needle track after five days; in those exposed for longer periods the organism was killed. Growth was abundant in the controls.

A drop of a liquid culture was introduced into each of eight tubes of nutrient broth, and a stream of SO_2 passed through the tubes for periods varying from 15 to 60 seconds. There was no clouding in any of these.

Reduced pressure. A set of tube cultures was prepared and sealed in Bulloch's apparatus; then the air was exhausted as completely as possible from the bell jar. In this experiment the oxygen was not absorbed.

At the end of four days there was only a slight growth in the glucose formate agar and in the formate broth tubes, but quite a good growth in the tubes containing ordinary nutrient agar and broth.

Effect of germicides. In connection with the spraying experiments a series of cultures was made to determine the susceptibility of the organism to various germicides.

In testing substances which could be added to nutrient broth without causing precipitates the procedure was as follows: A young broth culture was used, one which had been kept at 30° C. for 24 hours. From this ·1 cc. of the culture was dropped into each of a number of tubes of + 15 broth, and this amount did not cause any clouding of the medium. Into the tubes thus inoculated, each of which contained exactly 10 cc. of broth, varying quantities of a solution of the germicides were pipetted, about five tubes being used for each percentage.

After remaining in the incubator at 30° C. for 30 minutes a series of plates was poured from one tube in each group, the remaining four being returned to the incubator. The plates and tubes were kept under observation for a number of days until it was certain whether growth would take place or not.

Only a few substances could be satisfactorily tested in this way, and the results obtained with these may be tabulated as follows:

Germicide	Amount to retard growth	Amount to inhibit growth	Amount to kill organism in 30 mins.
Formalin	1 : 20,000	1 : 10,000	1 : 1,000
Copper sulphate	1 : 1,500	1 : 1,000	1 : 200
Phenol	1 : 1,500	1 : 1,000	1 : 100
Sodium nitrate	1 : 25	1 : 10	
Kainite	1 : 25	1 : 10	

Lithium sulphate in the proportions of 1 : 25 had no effect in retarding growth. I did not find any involution forms in cultures containing 4 % of this salt.

Substances which caused precipitates in nutrient broth were tested in a slightly different way. Tubes were prepared containing sterile distilled water with various percentages of the germicide. Into these ·1 cc. of a 24-hour old broth culture was pipetted and they were allowed to stand at 30° C. for thirty minutes. Plates were then poured as from the beef broth tubes in the previous experiment and these were kept under observation for several days.

The results obtained were as follows:

Germicide	Amount to kill large percentage of organisms in 30 mins.	Amount to kill all organisms in 30 mins.
Mercuric chloride	1 : 20,000	1 : 10,000
Hycol	1 : 2000	1 : 1000
Formalin	1 : 2000	1 : 1000
Cyllin	1 : 2000	1 : 1000
Lysol		1 : 400
Copper sulphate		1 : 400
Potassium permanganate	1 : 400	1 : 350
Iron sulphate	1 : 100	1 : 25
Iron sulphide	1 : 75	1 : 25

From this table it is evident that the organism is most susceptible to mercuric chloride, but it was not considered advisable to make use of this substance for spraying owing to its highly poisonous nature.

The results of spraying with Hycol have been given in another part of the paper.

Action of sunlight. The organism is not sensitive to exposure to light. It grows well in the diffused light of the laboratory, though not so rapidly as in the dark.

A series of thinly sown plates were exposed to direct sunlight, any undue heating being prevented by placing over each a glass basin containing about 2 cm. of a 2 % solution of potash alum. The number of colonies on plates exposed one to four hours was not appreciably less than the number developed in the control plates; there was a slight diminution in numbers in those exposed for five hours. This experiment was conducted in winter during the month of July.

Four cover glasses on which a film of bacilli had been dried were exposed to bright sunlight for one, two, three and four hours, then dropped into tubes of nutrient broth. Those exposed for one and two hours clouded the broth in 24 hours; those exposed three and four hours in 48 hours.

Thermal Relations.

The apparatus used for the determination of the thermal death point and figured below was adapted by Mr Ensor of the Public Works Department from a Hearson vacuum embedding bath intended for working with gas; it answers the purpose admirably, and regulates within one-tenth of a degree.

I am indebted to Mr Ensor for the following brief description of the apparatus and for the figure explaining the electrical connections:

Constant temperature water bath. The apparatus consists of the water bath of a Hearson vacuum embedding bath (*A*, diagrams 1 and 2). This is electrically heated by means of two eclipse elements (*L*, diagram 3) fixed to the bottom of the bath. The elements are connected in series on 250 volts.

For automatic regulation of temperature a switch (*B*, diagrams 1, 2 and 3) actuated by a capsule (*J*, diagram 3) is provided, this makes or breaks the heating circuit at the required temperature. A lamp (*K*, diagram 3) is joined across the switch contacts to prevent sparking.

There is also a tumbler switch (*C*, diagrams 1, 2 and 3) by means of which the temperature can be controlled by hand if desired.

The water is continually stirred by means of a paddle (*D*, diagrams 1, 2 and 3) driven by a small motor *E* fixed above the bath.

In order to cut down the speed of the motor to about 60 revolutions per minute a lamp (*H*, diagram 3) is connected in series. The speed can be varied by placing lamps of different candle power in the lamp holder.

The motor is started and stopped by the switch *F*.

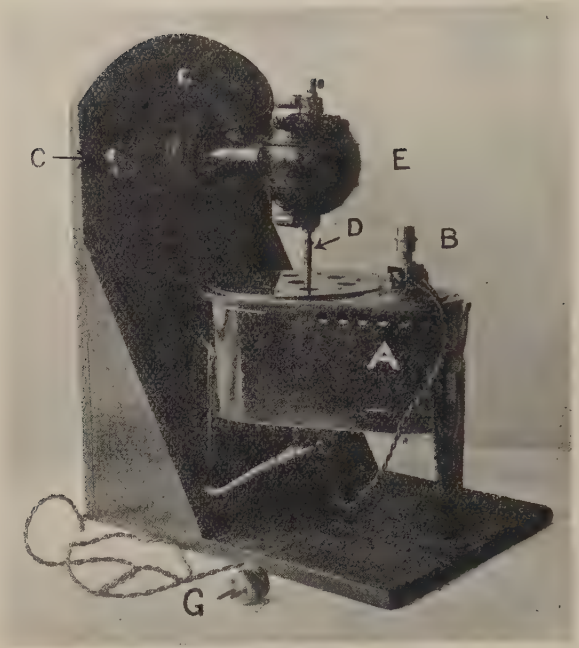

Fig. 1

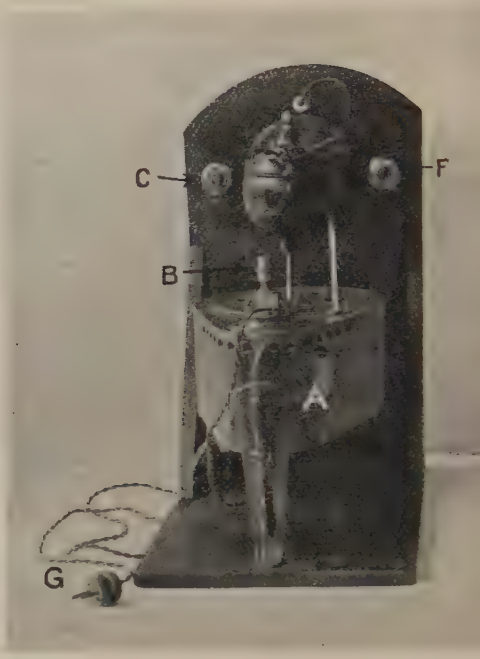

Fig. 2

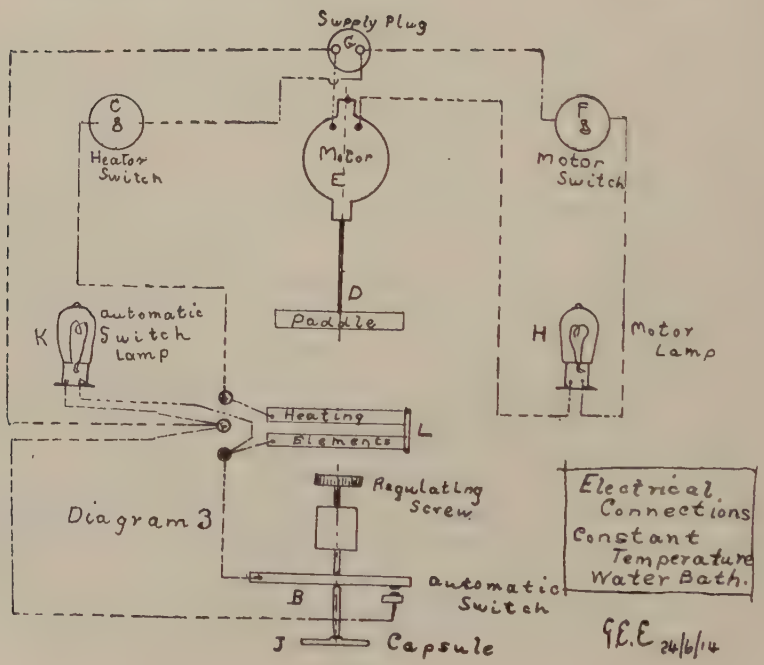

Fig. 3

The apparatus is connected to the nearest supply plug or lamp holder by means of a flexible cord and plug or adapter.

The tests were made with specially uniform tubes of thin glass, about 18 cm. in diameter and containing exactly 10 cc. of nutrient broth. Into each of these was introduced a loopful of a 24-hours old culture in nutrient broth, and they were placed in the water bath for ten minutes; at the end of the ten minutes the tubes were plunged into cold water to reduce the temperature and were finally placed in the incubator at 30° C. with a number of control tubes. The thermal death point was found to be 60° C. when determined by this method.

A second series of experiments was undertaken in order to determine the death point of the organism in a dry condition.

A number of sterile cover slips were smeared with a suspension of an agar streak in a normal saline solution. These were dried in a sterile petri dish and then exposed for ten minutes to the heat of a drying oven. At the end of this time they were taken from the petri dish with a pair of sterile forceps and dropped into tubes of nutrient broth. The broth clouded when inoculated with cover slips exposed to temperatures of 120° C. and under, but remained clear if the cover slips dropped into it had been exposed to a temperature of 125° C. or over. The thermal death point of the organism in a dry condition, therefore, lies between 120° C. and 125° C.

The organism can grow through a wide range of temperature if it is provided with sufficient moisture. It grows very slowly at 5—6° C. and also at 45°. At the latter temperature nutrient broth was feebly clouded at the end of 48 hours. The optimum temperature is about 30° C.

Desiccation.

The organism will not grow without a fair amount of moisture, and its growth is most vigorous on a wet medium and in a saturated atmosphere; on the other hand, it is very resistant to desiccation and remains alive for a long time in a dry condition.

A number of diseased leaves were dried in the air in the laboratory, being merely protected from dust. They were received on the 9th of June, 1913, and from that date cultures were made from them at intervals of a month. On the 9th of June, 1914, after the leaves had been drying for one year a vigorous culture was obtained; it has not yet been ascertained how much longer the organism retains its vitality on dried leaves.

An experiment was also conducted to test the ability of the bacillus to withstand desiccation on glass cover slips.

A 48-hours old culture on nutrient agar was suspended in a normal saline solution and a number of sterile cover slips smeared with the suspension were put in a sterile petri dish to dry.

A Hempel desiccator containing sulphuric acid was prepared, the dry cover slips transferred to a sterile ventilated capsule, and placed in the desiccator. The latter was then sealed up and partially exhausted. At intervals cover slips were removed from this apparatus with sterile forceps and dropped into tubes of nutrient broth. After 40 days drying a culture of the organism could be still obtained from the cover slips; at the end of that time the experiment was discontinued.

Resumé of Salient Characters.

Bacillus mangiferae n. sp. An organism causing a disease of the leaves and fruit of the mango (*Mangifera indica*); it attacks the parenchyma, causing black angular spots on the leaves; and on the fruit discoloured roughened areas often accompanied by deep longitudinal cracks.

A short motile rod with rounded ends averaging $1{\cdot}5 \times {\cdot}6\,\mu$; usually single, chains formed in pellicle on liquid media; motile, with 2—8 long, peritrichiate flagella; no spores observed; capsules in ring above liquid media; involution forms (?) in broth containing high percentages of NaCl; stains readily with usual stains and by Gram's method.

Forms shining yellowish colonies on nutrient agar, undulate at 25° C.; liquefies gelatine; clouds nutrient broth forming ring pellicle and sediment; grows readily on blood serum, but does not cause liquefaction; grows slowly in milk finally causing coagulation, at 37° C. the casein is slowly dissolved. Grows on potato making a shining yellowish spreading growth over moist part of medium; the latter is not discoloured; heavy growth on beet and in beet juice, also in cabbage broth; slightly clouds Cohn's solution; no growth in Uschinsky's solution or on starch jelly made with Fermi's solution.

No gas formation in carbohydrate media, but medium becomes slightly more acid. No formation of diastatic or invertin enzymes, small percentage of alcohol in dextrose broth; nitrates reduced, tolerates up to 8·75 % NaCl; reduces litmus and several other colouring matters; indol in media containing peptone but no phenol.

Aerobic, facultative anaerobe; very sensitive to action of mercuric

chloride, but less so to copper sulphate; not sensitive to sunlight; will not grow without moisture, but is not easily killed by desiccation; will grow in media of widely different reaction, optimum reaction about + 17 Fuller; maximum temperature for growth 45° C., thermal death point 60° C. Group No. 221. 2223532.

Summary.

1. A disease of mangoes, hitherto undescribed, has for the last few years been causing considerable loss to mango growers in the Union.

2. So far as can be ascertained the disease is not known outside South Africa, and it occurs in this country in the neighbourhood of Barberton and Warmbaths, in the coast region of Natal and at Delagoa Bay.

3. Dark angular spots are formed on the leaves which do not noticeably affect the general health of the tree, but serve as a source of infection for the fruit. On the latter the disease causes discoloured roughened areas and deep cracking; infected fruit is detached from the tree by the slightest air movement and falls rotting to the ground.

4. Infection is carried by the wind, and by rain dripping from infected leaves. Very few insects are found on the mango foliage.

5. Spraying experiments have been conducted which show that spraying with Bordeaux mixture, iron sulphide or Hycol is useless in checking the disease.

6. The cause of the trouble is a flagellate bacillus *Bacillus mangiferae* n. sp. It invades the parenchyma, wedging apart and killing the cells and causing gummosis; lignified tissues are not touched.

7. The organism is described in detail and a resumé is given of its salient characters.

Acknowledgment.

I wish to acknowledge my indebtedness to Mr I. B. Pole Evans, Chief Division of Botany, in whose laboratory the work was done, for many helpful suggestions; and to Mr E. E. Ensor of the Public Works Department for adapting the apparatus described in connection with the thermal death point determinations.

The names of the varieties of mangoes grown in this country were furnished by M. D. d'Emmerez de Charmoy, of the Department of Agriculture, Mauritius.

I have also to thank Messrs Winter Brothers, of Barberton, who placed their orchard at our disposal for the spraying experiments, and who have repeatedly supplied us with mango fruit for experimental purposes.

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EXPLANATION OF PLATES.

All drawings were made with the aid of the camera lucida, except figures *c*—*h* of Plate XI, which were drawn freehand as explained in the text.

Plate I. Three colour photograph of small branch bearing diseased fruit. (Reproduced, from nature, by the Government Printer, Pretoria.)

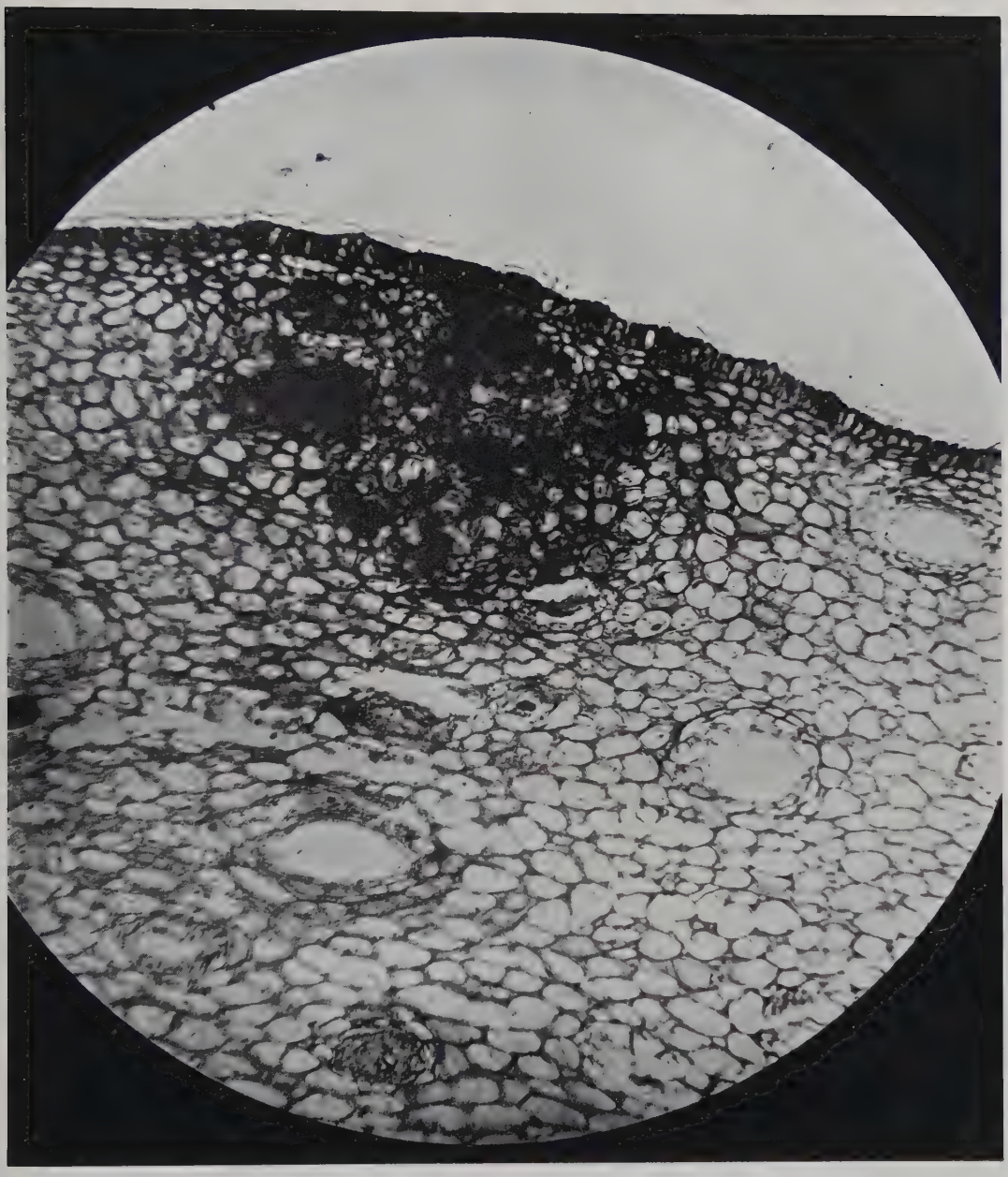
Plate II. Mango leaves showing the dark coloured, angular spots, characteristic of the disease.

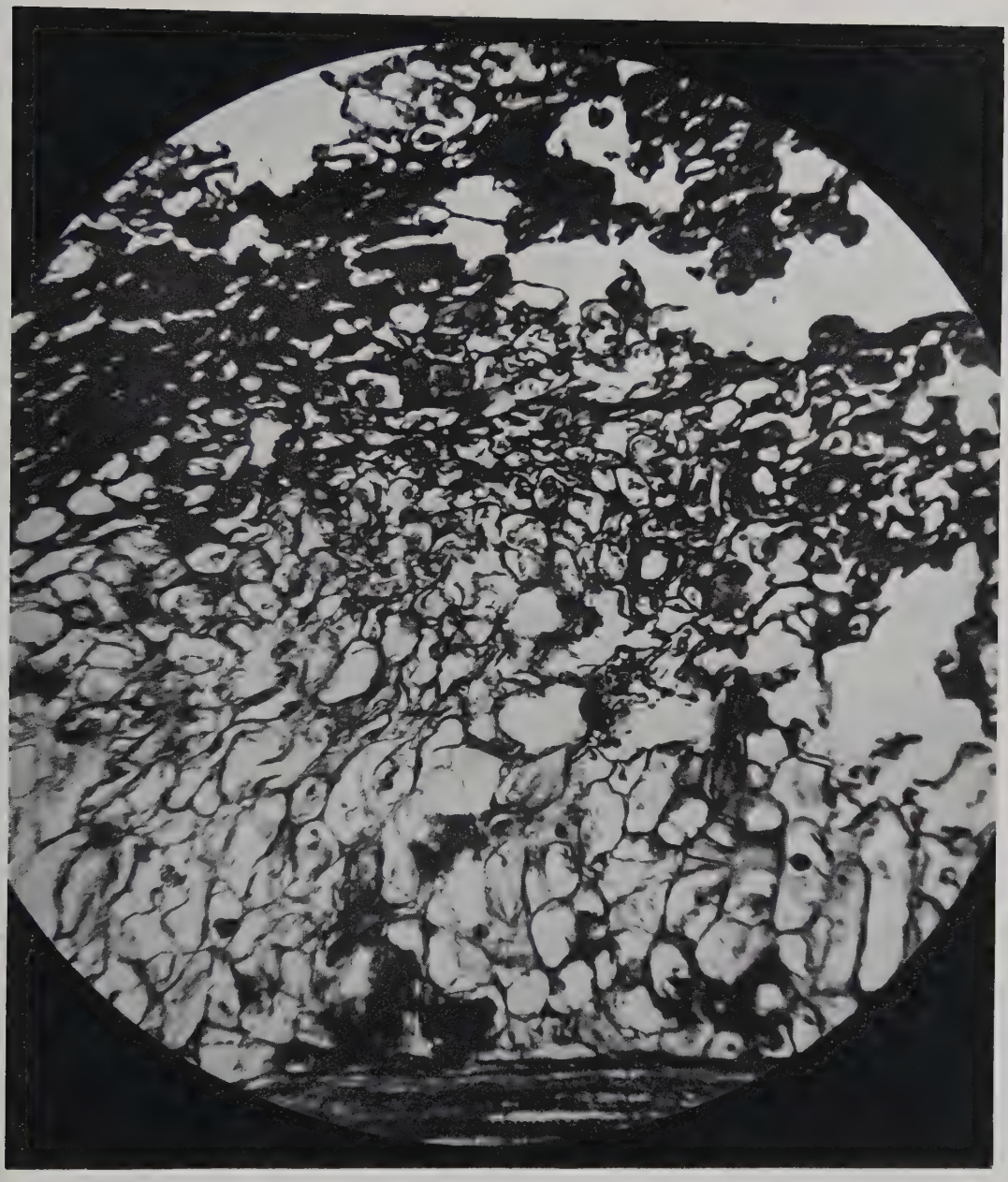
Plate III. Small branches which have been infected through hail injuries. A small portion of bark has been removed from the left-hand branch to show the discolouration of the underlying tissues.

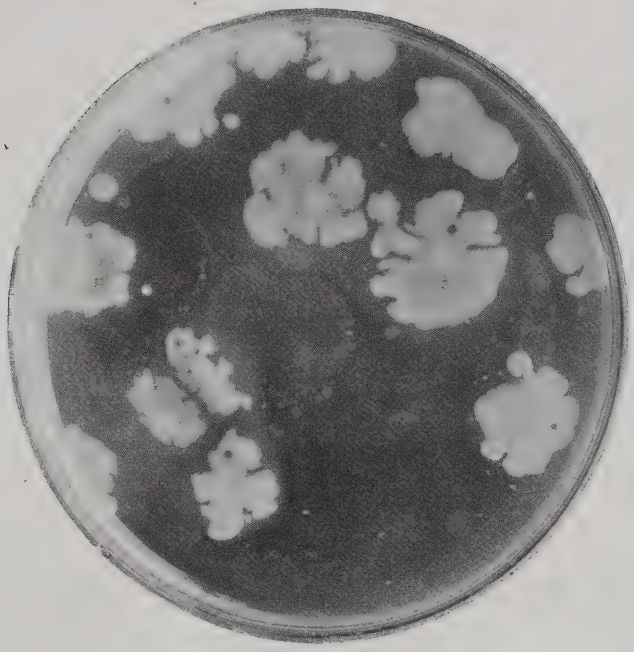
Plate IV. Half-grown fruit showing discoloured roughened areas caused by *Bacillus mangiferae*.

Reproduced, from nature, by the Government Printer, Pretoria

Mango fruits, attacked by Bacillus mangiferae







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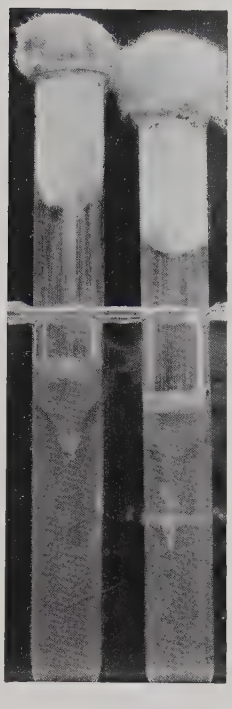

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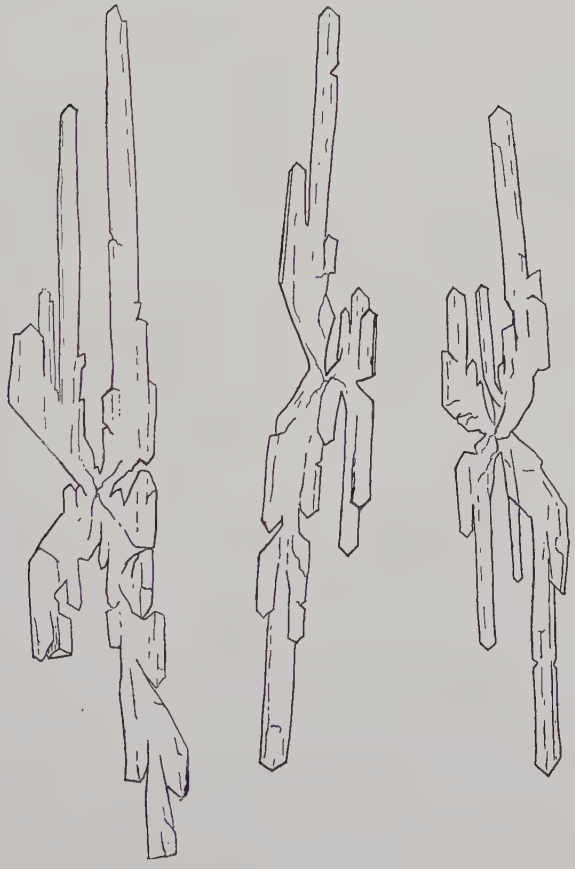

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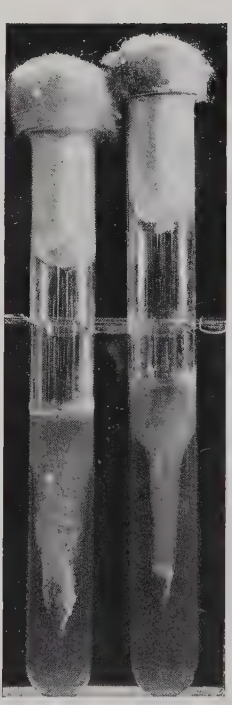

d

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d
1.25 p.m.

e
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f
1.55 p.m.

g
2.10 p.m.

h
2.17 p.m.

i
2.25 p.m.

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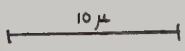

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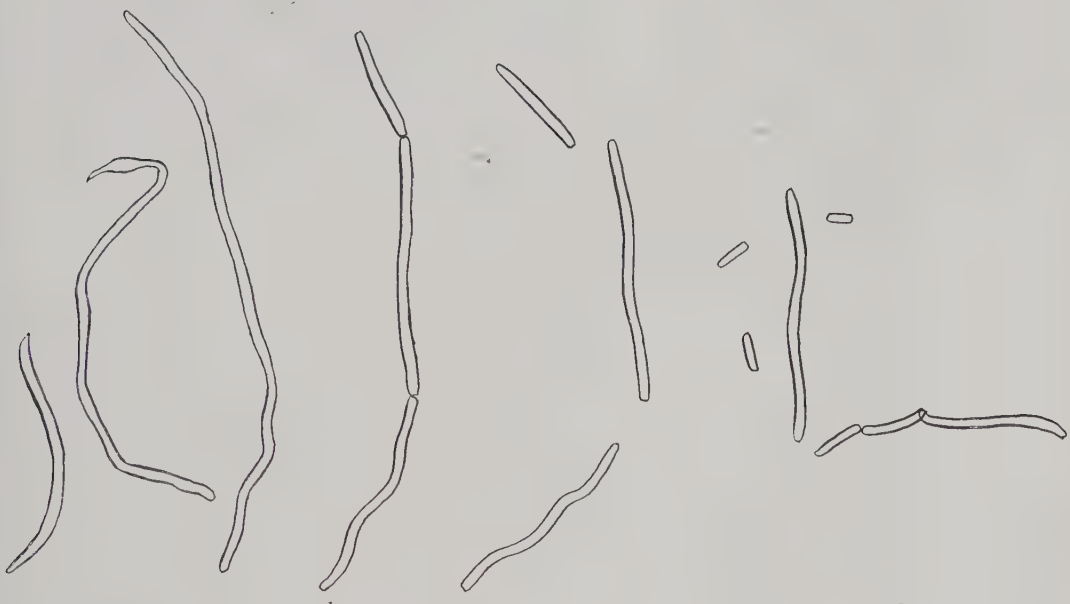

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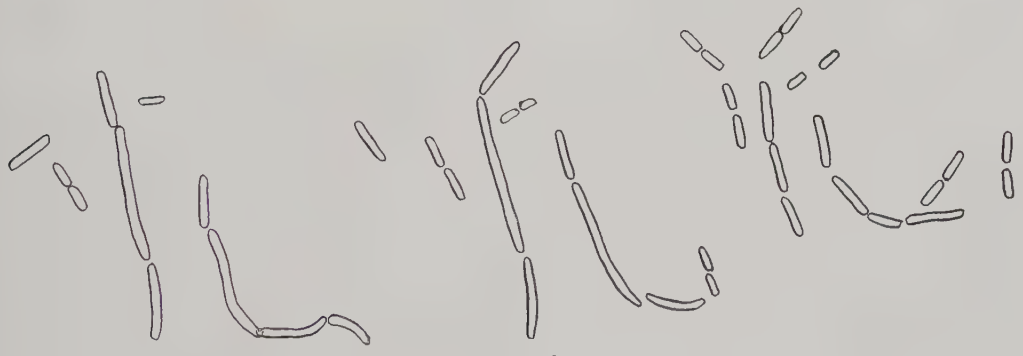

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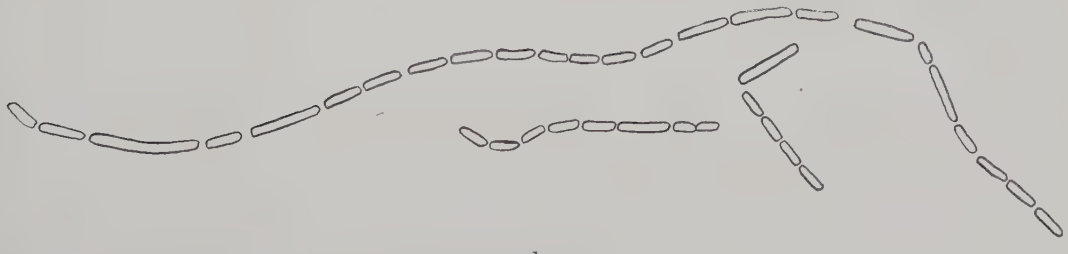

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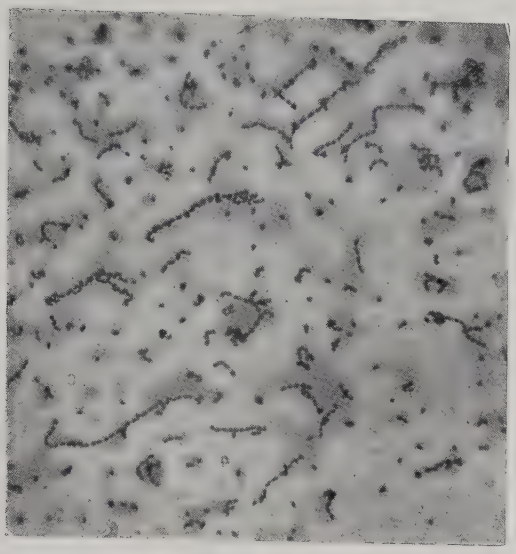

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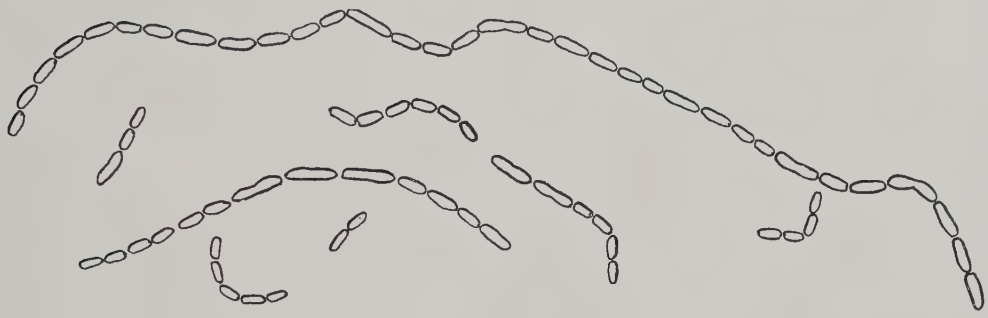


b

e

d

Plate V. Mangoes in a more advanced stage of the disease; deep longitudinal cracks have formed.

Plate VI. In this case the whole inflorescence has been destroyed; the one remaining fruit fell off as the photograph was taken.

Plate VII. Photomicrograph of section through fruit, showing an early stage of the disease. The dark area consists of crushed, discoloured cells, with masses of bacilli in the intercellular spaces. (Photographed with Zeiss' achromatic objective D, and compensating ocular No. 6.)

Plate VIII. Photomicrograph of section through fruit in a more advanced stage of the disease. The superficial cells are crushed and dead; there are very few bacilli in this region. They are very numerous in the more deeply seated tissues, a small fibro-vascular bundle at the foot of the plate is surrounded by them, but untouched. Same magnification as Plate VII.

Plate IX. Detail from Plate VIII., in the neighbourhood of the fibro-vascular bundle. The bacilli can be clearly seen in the intercellular spaces, wedging the cells apart. (Zeiss oil imm.; obj. 2 mm., 1·4 app.; comp. ocular No. 6.)

Plate X. Photomicrograph of section through diseased spot on leaf. The increase of thickness due to the swelling of the disorganised cells is very evident; two small vascular bundles in the field have not been attacked. The organism has invaded the tissues from the underside of the leaf, the palisade cells still being intact. There are no stomata in the epidermis of the upper side of the leaf. (Same magnification as Plate VII.)

Plate XI. (*a*) Plate culture of *Bacillus mangiferae* photographed after five days at 25° C.

(*b*) Crystals from plate culture in +15 nutrient agar, 10 days old. Photographed from a drawing made with camera lucida, and Zeiss achrom. obj. AA., ocular No. 1.

(*c*) Stab cultures in nutrient gelatine photographed after 20 days at 20° C.

(*d*) The same culture seven days later.

Plate XII. *a*—*o*. Development of a small colony as observed on agar hanging block. For fuller explanation see text. (Zeiss 1/12 obj. oil imm. and No. 12 compensating ocular.)

Plate XIII. *a*—*h*. Stages in the segmentation of long threads developed in nutrient broth containing 8·5 NaCl. Full explanation in text.

Plate XIV. (*a*) Photomicrograph of chains formed in pellicle on nutrient broth, eight days at 30° C., stained with MacConkey's capsule stain.

(*b*) From same preparation as above, drawn with camera lucida, Zeiss oil imm. obj. 1/12 and compensating ocular No. 12.

(*c*) Bacilli from 2 % dextrose broth, three days at 30° C. (Paraboloid condenser, Zeiss apochrom. obj. 3 mm., apert. 0·95, compensating ocular No. 18.)

(*d*) Capsuled rods from ring above nutrient broth, 15 days at 30° C., MacConkey's capsule stain. Zeiss oil imm. obj. 1/12, comp. ocular No. 12.

(*e*) *Bacillus mangiferae* with flagella, from culture on nutrient agar, 20 hours at 25° C. Stained by Ellis' modification of Loeffler's method. Same magnification as (*d*).

[Note by Editor. It has been necessary to reduce the size of some of the figures; for which allowance must be made in the estimate of magnification.

Plates VII, VIII, IX and X have each been reduced by one-eighth.

On Plate XI, figs. *c* and *d* have been reduced by one-eighth.

Plate XIII, all figures reduced by one-sixth.

Plate XIV, figs. *c* and *e* reduced by one-fifth.]

Botanical Laboratories of the Union of South Africa, Pretoria.

TWO SCOLIID PARASITES ON SCARABAEID LARVAE IN BARBADOS.

By W. NOWELL, A.R.C.S.

(*Imperial Department of Agriculture for the West Indies.*)

(With Plate XV and 1 Text-figure.)

As introduction a word should be said regarding the incidence of the rainfall in Barbados and the agricultural operations determined by it, since these have a bearing on the life of the insects to be considered. There is no definite separation into dry and wet seasons, but generally speaking the first five months of the year are characterised by drought, broken now and then by more or less heavy showers; from late May or early June onwards these are supplemented by the occurrence of very occasional falls of two or three inches of rain, producing what may by contrast be called the rainy season. The canes are planted towards the end of this season, about December, and are reaped in March and April, some sixteen months later. After the reaping season, as showers permit, various other crops—among them sorghum and maize—are planted, and these have often accomplished a fair amount of growth by the time that the heavier rains bring in the period of greatest insect activity.

Two beetles of the family Scarabaeidae occur in Barbados. *Ligyrus tumulosis* Burm., a Dynastid, is universally distributed and very abundant. Its larvae live in soil rich in decaying vegetable matter. They occur in very large numbers in pen manure and are to be found at the base of the heaps of dead sugar-cane leaves or cane stumps which are made up at the end of the reaping season. They are not known to attack living plants. The beetle has no definite seasonal distribution, but the adults are capable of remaining for a considerable time in the soil, from which they emerge in large numbers after rain.

Phytalus smithi Arrow, a Melolonthid, though common, is much less noticeable owing to the fact that the adults are very rarely attracted

to light. They emerge in large numbers after the May rains and may be found feeding at night on the foliage of various field and garden plants. At other seasons of the year they are very seldom seen, though there is evidence that they occur in small numbers. The larvae attack the roots of many plants, and in gardens are very destructive to rose bushes, but it is as pests of sugar-cane that they are of special importance. Their occurrence in destructive numbers in the Barbados cane-fields is rare and seems confined to small and shifting areas, but in Mauritius, into which island it seems highly probable that they were introduced in sugar-cane stools from Barbados, great and continuous damage is being caused over an ever-widening area.

While investigating root grubs of sugar-cane at Spencers Plantation towards the end of the year 1911 the writer noticed in the soil beneath and about the roots of sugar-cane small numbers of brown cocoons. These proved to contain pupae of a black Scoliid wasp, which was bred out. When forwarded later through Mr G. A. K. Marshall, then Secretary of the Entomological Research Committee, it was identified at the British Museum as *Tiphia parallela* Smith. The specimens then in the British Museum collection, which were females only, had been obtained from Brazil.

Both sexes are shining black in colour, with greyish pubescence on the legs and grey fringes at the joints of the abdomen. The wings are lightly tinted with brown. There is very considerable variation in size among the females, the range in length (excluding the antennae) in a representative collection being from 15 mm. to 9 mm. The males range from 9 to 6·5 mm., excluding antennae and anal spine. The males are readily distinguished by the presence of this spine, which projects from the tip of the abdomen; its rigidity and stoutness sufficiently prevent confusion with the sting of the female, and it has a marked upward curve.

By examination of the remains adhering to the cocoons the writer was able to determine that the host was the larva of Phytalus.

After rain in early June the adult wasps, male and female, were found in large numbers in the same locality feeding on the honeydew on aphis-infested sorghum. They were observed in greatly fluctuating numbers until about the end of September, and it was noticed that towards the end of the period there was a large preponderance of males. In 1913 large numbers again occurred in June, in practically the same place. That their emergence is not confined to the wetter months is proved by the finding at all periods of the year of newly parasitized

larvae. Possibly there is some check to emergence during dry weather, though experiments in the insectary have not supported the supposition. Of 21 laboratory-reared examples, each of which had pupated in earth in a separate Petri dish, 11 which were kept air-dry averaged 35 days, and 10 which were kept moist averaged 36 days in the cocoon. The periods ranged between 32 and 40 days, with one exception in each case: one of those kept dry emerged in 47 days, one of those kept moist in 45 days. More observations are needed, but partly at any rate the finding of large numbers in the wetter months must be ascribed to the opportunities for collection afforded by the attraction of the honeydew. No instance of their visiting flowers has been observed. Of the distribution of Tiphia about the island little is known, and that mainly of a negative character. An outbreak of Phytalus, small in area but rather severe, in a cane-field at Waterford Plantation, 10 miles from Spencers, was examined in May 1913, and amongst a large number of cane stools dug up only one Tiphia cocoon was found. The Manager of Spencers Plantation states that a similar outbreak of Phytalus took place some three years back in the fields where Tiphia is now so common. It would appear that we have quite sufficient room in the small area of Barbados for the typical sequence of a local outbreak of a pest followed by the migration and ultimate preponderance of its parasite.

Under these circumstances the percentage of parasitism depends upon the stage which has been reached in the local cycle. In the well-developed example at Spencers the number of cocoons and parasitized grubs found during extensive digging amounted to some thirty per cent. of the total number of grubs and cocoons found. There being several generations of Tiphia to each generation of Phytalus; this figure must be much below the actual one, since no account was taken of the large number of empty cocoons representing recent generations of Tiphia, nor does it include the number of grubs which would still have been attacked before pupating.

Information as to the life history of the parasite was first obtained from discoveries of early stages in the field. Later the stocking of Wardian cases for an attempt to introduce it into Mauritius afforded further opportunities, especially for watching the behaviour of the adult wasps, while it has recently been found possible to carry through successive generations in the insectary.

The largest number of eggs actually obtained from one female in captivity is six, but an examination of the ovary tubes seems to indicate an egg capacity of at least seventy.

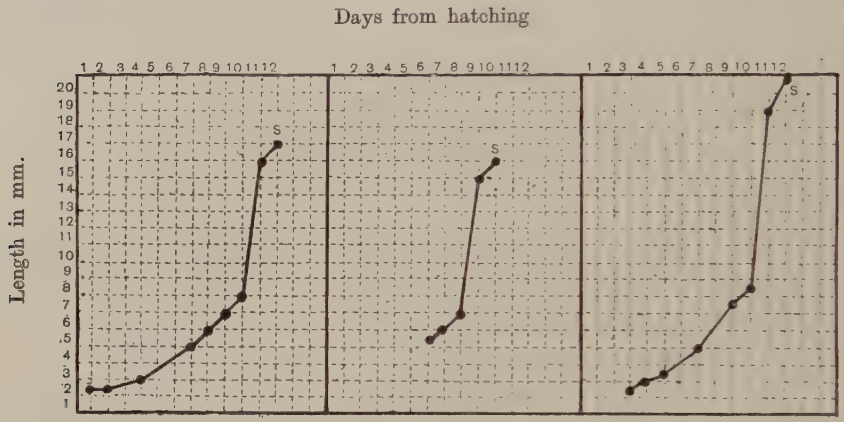
The laying female may be seen in the field running quickly over the surface of the soil, her antennae vibrating rapidly all the while. As she obtains the required indication she commences to burrow, shuffling forward with her legs and appearing to use her head to separate the particles of soil.

Tiphia has never been induced by the writer to take any notice of a grub on the surface, but the method of attack was seen in the case of a half-buried grub and closely resembled that of *Campsomeris dorsata*, to be described in detail later. Neither Tiphia nor Campsomeris will lay on a grub close to the surface, but after stinging it into quiescence each proceeds to burrow under it and drag it down. The Tiphia mentioned above took several minutes in getting her prey out of sight, during which it recovered activity after the first sting and received a second.

The egg is laid transversely in a fold of the dorsum of the thorax, and is firmly agglutinated throughout its length by a cement which is colourless at first but eventually becomes dark brown. The egg measures a little over a millimetre in length. It is quite white when first laid but soon darkens slightly. In no case under natural conditions has more than one perfect egg been found on one grub. Occasionally a grub has been found in the field with an egg or larva upon it and in addition to this a brown elongated spot has occurred, apparently marking the position of a previous egg. In the insectary, where the operations of several females were confined to a limited number of grubs in the same flower-pot, such cases were very common and the remains of two eggs in addition to the living one were sometimes found on the same grub. It appears somewhat probable that when a wasp about to deposit her egg finds one already there she destroys it.

The egg stage occupies 5–6 days. At the end of this time the membrane splits transversely close to one end, which then forms a cap hinged on its dorsal margin. From the opening thus made the head and one or two additional segments of the larva are extruded. While thus situated the larva punctures the skin of the grub with its mandibles. The perforation is very minute and is very little enlarged during the remainder of the life of the host. The head of the larva is closely applied to the hole, but is not pushed into it and is quite free to move. Whether in feeding there is an actual suction by the larva, or whether the fluid simply wells out from the wound and is imbibed from the surface, was not determined. Continuous movements resembling peristalsis may be seen within the body of the parasite. On

the day following that of hatching the egg membrane splits dorsally along its length, and the back of the larva is revealed, but the larva still firmly adheres by its ventral surface to the egg membrane below, thus retaining its attachment to the host. The successive moults are accomplished in a similar way: the skin splits along the back and gradually recedes down the sides of the larva as the latter increases in circumference. The number of moults was not determined with absolute certainty; in addition to that which occurs on passing into the pupal stage two were observed during the time that the larvae are attached to the host, occurring in normal examples 3–4 days and 7–8 days after hatching.



S indicates spinning

Growth in length of three Tiphia larvae

The increase in length of three typical examples is shown in the subjoined diagram. Until about the tenth day from hatching growth is steady and regular, this being the period during which the host remains alive, or at least, retains its normal shape and turgidity. Up to the last day or two of this period it is quite active, burrowing through the soil and in some cases feeding. On the last day it is without movement, and the large anal segment has usually become translucent. Until this stage is reached the parasite has retained its original attachment, becoming considerably arched as it increases in length, and hanging to one side across the back of the host like a firmly stuffed pillow. It now pushes out its long tapering neck, hitherto concealed, and attacks the actual tissues. It usually begins behind the head, eating a large hole into the thoracic segments of its now collapsed and

detached victim. The whole body is cleaned out in a few hours, only the shrivelled skin, with the feet and the hard brown integument of the head, remaining. The increase in size during this final stage of feeding is remarkable, the linear proportions being more than doubled.

The larva proceeds to spin up *in situ*, the long neck facilitating the attachment of the thread to different points in the wall of the cavity previously occupied by the host. A very loose fluffy outer covering is first formed, and within this a firm-walled, extremely tough, elongate-ovate inner envelope. The whole cocoon is whitish at first, but darkens to a tawny brown.

The process of spinning, so far as it can be externally watched, occupies about 24 hours.

The time spent in the cocoon, as observed in the insectary, is usually from 32–40 days. Only in three instances in the writer's experience has this been exceeded, in which cases periods of 45, 47, and 56 days were reached. Excluding these, the average of 22 carefully recorded examples was 35 days.

The wasp emerges by biting an irregular hole through the cocoon near one end, generally the larger one. It may be heard rasping away the envelope at that point with its mandibles an hour or so before its head appears.

Both sexes take food, if it is available, immediately after emergence. In captivity cane syrup, sugar and water, and honey are readily accepted.

Copulation has been observed both in the field and in captivity. If, however, a virgin female is confined over soil containing Phytalus grubs she will proceed to lay fertile eggs. The experiments which have established this were carried out with females reared separately in small Petri dishes. Phytalus grubs, examined under a dissecting microscope to make sure that they were free from eggs, were buried in soil mixed with a few fibrous roots in large flower-pots, made with a bulging rim to fit closely round the base of large lantern glasses. The top of each glass was covered with tissue paper held tight by a cardboard ring. A hole was punched through the paper with a pencil, the female (slightly chloroformed) introduced, and the hole closed with gummed paper. Difficulty in obtaining grubs limited the experiment, but some fifteen eggs were obtained from five females. All hatched and produced normal larvae. Owing to external circumstances only three adults were secured from these eggs, all males.

In view of experiments in introducing this or other species of Tiphia for the control of Melolonthid grubs in other West Indian Islands and perhaps elsewhere it may be of value to give the results of the writer's experience in Barbados. The most obvious procedure is to dig up and transmit the cocoons, but success has not ensued from the practice of this method. Large numbers of cocoons have been dug out under the writer's personal supervision. They have been exposed as little and handled as carefully as possible, kept on or in soil or in various receptacles, in different degrees of moisture. Always the number emerging has been disappointingly small, with no observed indication of the cause of failure. Under each condition tried, except when the cocoons were completely buried, a small percentage emerged, while the rest of the cocoons when opened showed larvae, pupae or imagos dead and dry. Buried cocoons failed altogether. It seems most probable that mechanical injury is inflicted in the disturbance involved in digging up and transporting the cocoons. The tenderness of Lepidoptera at the same stage will occur as an analogy to the entomologist. It scarcely needs to be added that shipment of such cocoons, in cool storage or by parcel post, has not been attended with success.

The method by which living material was eventually landed in Mauritius was as follows: Wardian cases with a sugar-cane stool established in each were stocked with healthy Phytalus grubs, and Tiphia imagos, male and female, were introduced and fed with syrup and water. Many of the grubs were later found to be parasitized, others had completed their development and commenced a new generation of Phytalus in the cases. Fresh grubs were introduced as they were obtained up to the date of shipment. The only information I have received was that from this material wasps were obtained in Mauritius which laid on Phytalus grubs in the insectary there. For introductions where the time taken in transit does not exceed the length of the life cycle of Tiphia it would be sufficient to have a number of boxes containing grubs in soil, to cover each for a few days with a cage of mosquito netting within which Tiphia imagos could be confined, and then to remove the cage and transmit simply the boxes with the parasitized grubs. Probably success might be attained in sending cocoons, provided these had been formed in the insectary in small boxes of soil, matchboxes say, so that they could be sent with a minimum of disturbance. In any case, unless the indication that the parthenogenetically produced wasps are males should fail to be confirmed by further experiments, it will be necessary to send a sufficient amount of parasite material to

give a reasonable chance of males and females emerging about the same time.

The second Scoliid to be described, *Campsomeris* (*Dielis*) *dorsata* (Fab.), is found in Barbados throughout the year. The wasps occur very abundantly on flowers at frequent and apparently irregular intervals, sometimes both sexes together, sometimes with one or the other greatly preponderating. Their visits to flowers cease shortly after mid-day. The males have a curious habit, so far observed only in the afternoon, of collecting in large numbers on any convenient piece of vegetation, such as a grass stem or a yam vine, forming an assembly like a swarm of bees. The writer is quite at a loss to account for this habit, which occurs in several other Fossorial wasps met with in the West Indies.

The sexes are very different in appearance. The male is slender, 12–17 mm. in length. The dorsal surface of the abdomen is striped laterally with yellow and black, the ventral surface is blue-black; the second (apparent first) abdominal segment is considerably smaller in circumference than the remainder. A narrow yellow band bordered with black makes an almost complete circle on the dorsum of the thorax at the level of the wing insertions and is repeated behind. The antennae are cylindrical, about 8 mm. long.

The female is stouter and has a length of 14–24 mm. The colour is a uniform shining black with the exception of a brick-red blotch which covers the dorsal aspect of segments three and four of the abdomen, divided only by a black line at their junction. The wings are of a deep metallic blue. The antennae are about 4·5 mm. long. There is considerable greyish pubescence on the thorax, legs, neck and waist, and on the front of the head.

The notable range in length observable in each sex of both Tiphia and Campsomeris is not a regular variation about a central mean. The larger sizes are more abundant. The tendency seems to be towards the attainment of the maximum for the sex, limited by the nutrition available from the single larva of the host. Attention has not been specially directed to the question as to whether there is any tendency to the selection of large grubs by the laying female, but quite small grubs have occasionally been noticed to be parasitized. Observation has seemed to show, quite definitely in the case of Ligyrus, that in a continuous breeding place small larvae are much less common at any one time than those approaching full size, due presumably to the earlier stages of growth being passed over more rapidly, which, granted the

influence of nutrition, would sufficiently account for the relative infrequency of the smaller sizes of the parasite. The whole question is perhaps worthy of direct investigation.

The life history of this wasp was worked out during 1912 at Spencers Plantation. Since it had not at that time been found by the writer in connection with Phytalus, the probabilities pointed to Ligyrus as its host, and a search revealed, associated with Ligyrus in pen manure, an abundance of parasitic larvae and cocoons which turned out to be those of Campsomeris.

When pen manure, containing large numbers of Ligyrus grubs, was being put out in the fields and buried alongside the young clumps of sugar-cane in February 1913, large numbers of the wasps, male and female, were seen in the same fields; the males were darting rapidly about just above the ground, the females were flying from one cane clump to another and alighting to search the surface of the soil over each place where the manure was buried. Many were seen to burrow rapidly out of sight in such situations. They remained below for very various lengths of time, and in most cases digging at the spot where the wasps had burrowed revealed, at a depth of from six inches to a foot, a larva of Ligyrus alive but stupefied and with an egg upon it.

A healthy larva of Ligyrus was dug up and placed on the surface of the soil near to a hunting female Campsomeris. The latter quickly took notice of it, and a struggle ensued between the two which lasted about five minutes. The wasp, before she would use her sting, manoeuvred for a hold which would enable her to plant it in the right place, which proved to be a spot between the legs of the larva, as near as could be made out between the third pair. Three times when the wasp faced the grub for this purpose some part of her head or its appendages was seized between the mandibles of the grub. When she finally succeeded in inserting her sting the grub immediately relaxed and became quiet. The wasp then proceeded to burrow under it, and after loosening the soil, she pulled it under. This was about 5.15 in the evening.

The place was marked and visited early next morning, when the grub was found buried ten inches deep, with an egg upon it. The wasp, in an exhausted condition, was turned up at the same time. There was no manure within several feet of this place, so that it is unlikely that a second grub was mistaken for the first.

The egg of Campsomeris is attached by one end about the middle of the ventral surface of the grub. It is easily separated and the resulting

larva is not attached to its host. The difference from Tiphia in these points is no doubt correlated with the fact that the Ligyrus grub does not recover its activity after being stung by Campsomeris, but remains in a quiescent condition, making only slight movements of its legs when disturbed. Since, as will be mentioned later, Phytalus is occasionally successfully parasitized by this wasp, it appears likely that its sting has the same effect on the larva of that insect.

The development of the Campsomeris larva is very similar to that of Tiphia. The history of the specimen above mentioned may be continued as an example. The egg was laid during the night of the 13th or, more probably, early in the morning of the 14th of February. The further observations made are summarised below:

Date	Day	
Feb. 14	1	Grub with the newly deposited egg placed in insectary.
„ 15	2	Not hatched at 1 p.m.
„ 16	3	No observation.
„ 17	4	Egg found to be hatched when examined at 10 a.m.
„ 21	8	Parasite well grown; host still shows faint movements.
„ 22	9	Parasite growing fast; host doubtfully alive.
„ 24	11	Host quite dead, skin half empty; parasite more than doubled in length since last observation.
„ 25	12	Skin empty except for earthy matter in alimentary canal. Parasite disengaged itself during day and began to spin up.
„ 26	13	Cocoon getting well into shape at 9 a.m.

The actual date of emergence is not known. The cocoon was found empty on April 15th, the wasp having escaped unnoticed.

The duration of the egg stage was thus 3–4 days; the host remained alive 6–7 days after the parasite hatched; spinning began on the 9th day after hatching. The duration of the cocoon stage was not more than 48 days, probably some days less.

The cocoon of Campsomeris is easily distinguishable from that of Tiphia. The shape is cylindrical-oval. The outer envelope is not loose and fluffy as in Tiphia, but has a consistency like that of loose-textured tissue paper. It encloses the inner envelope somewhat slackly. The method of emergence is also different: the wasp cuts round the cocoon near one end and pushes to one side the neat cap thus formed.

Campsomeris is by far the most common of the flower-visiting Hymenoptera in Barbados, but although it is very plentiful the percentage of Ligyrus parasitized at Spencers has not been found to be large in the situations explored. Pond mud, of close texture and very

firm, is largely used in making up the pen manure, and seems to afford protection to large numbers of the grubs. As described above, when this material is more or less broken up by distribution in the fields, the wasps collect about it in a way that is not seen before it is disturbed.

In digging up cane stools in the fields an imago or a cocoon of Campsomeris was occasionally met with in the situations usually occupied by Tiphia, and in the extensive digging operations connected with the shipments to Mauritius it was definitely established by the examination of the skins attached to these occasional cocoons that Campsomeris is to a small extent parasitic on Phytalus[1]. The ratio of this parasitism to that due to Tiphia at the same time and place was calculated to be about one hundredth. The writer has never found Tiphia on Ligyrus.

A Rhipiphorid beetle, identified through the Imperial Bureau of Entomology as *Macrosiagon octomaculatus* Gerst., has several times been found to emerge from cocoons of Campsomeris, and has been taken on flowers of *Antigonon leptopus*, which are much frequented by the imagos of that wasp. No direct information has yet been obtained as to its method of parasitism, but it is probably that common to the order, the eggs being laid on or near flowers, the young larva attaching itself to a wasp visiting the flowers, and transferring itself when the egg of the wasp is laid. Numbers of a triungulin whose relations have not been ascertained were recently found on several Ligyrus larvae which bore eggs of Campsomeris.

So far observations have shown that Tiphia, which does not visit flowers, completely escapes this parasite.

The investigations described in this paper were carried out in connection with the Barbados Department of Agriculture, and are published by kind permission of Mr J. R. Bovell, I.S.O., F.L.S., F.C.S., Superintendent of Agriculture. The somewhat ragged state in which certain matters have been left is due to my transfer from that Department and the assumption of duties of a different nature. I am specially indebted to Mr A. A. Evelyn, of Spencers Plantation, without whose interest and co-operation the field work would have been impossible, and my thanks are due to my present colleague, Mr H. A. Ballou, M.Sc.,

[1] An earlier record of the parasitism of Campsomeris on Phytalus is contained in a letter from Mr G. A. K. Marshall to the Superintendent of Agriculture in Barbardos, mentioning the finding of one example at Spencers, in February 1912. This is the occurrence referred to in the Report of the Bureau of Entomology, 1914.

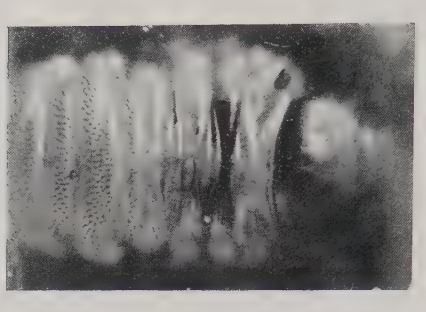


Fig. 1

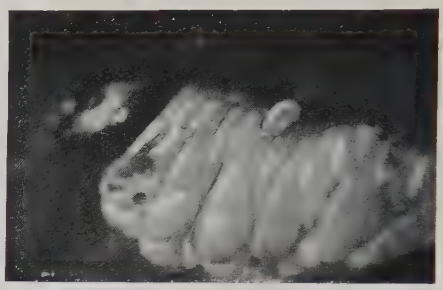


Fig. 2

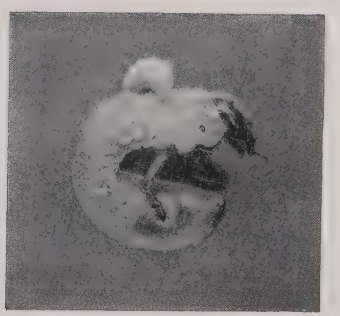


Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

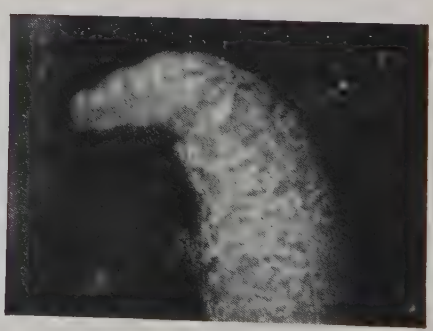


Fig. 8

Entomologist to the Imperial Department of Agriculture, with whom I have discussed various points arising in the course of the research and in the preparation of this paper.

EXPLANATION OF PLATE.

Fig. 1. *Tiphia parallela*: egg on Phytalus; × 4¼.
Fig. 2. „ larva three days old; × 4¼.
Fig. 3. „ larva eight to nine (?) days old; × 1½.
Fig. 4. *Campsomeris dorsata*: egg on Ligyrus.
Fig. 5. „ „ larva one day old.
Fig. 6. „ „ larva beginning to attack tissues of host; × 4¼.
Fig. 7. „ „ larva at end of feeding period; × 3.
Fig. 8. „ „ larva beginning to spin up; × 4¼.

(Fig. 3 from a photograph by H. A. Ballou; remainder by the author.)

PINK DISEASE OF PLANTATION RUBBER.

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(*Assistant Mycologist, Federated Malay States*).

(With Plates XVI and XVII and 11 Text-figures.)

Introduction.

There has been a considerable development of Pink Disease in Malayan rubber estates since 1912 and at the present time it is the disease which requires the greatest amount of attention in many districts. We have seen several estates in which 10 per cent. of the trees were affected and in a few neglected plantations in parts of the country where the disease was bad no less than 25 per cent. were attacked. During 1914 an opportunity was given us of investigating this disease in detail especially as regards plantation rubber and the present paper embodies the chief results of this investigation. Other information about the disease, which is of interest only to planters, is included by us (4) in Bulletin 21 of the Department of Agriculture, Federated Malay States.

Distribution and Hosts.

Pink Disease is caused by a fungus which was named *Corticium javanicum* in 1901 by Zimmermann (17) who investigated it in Java with special reference to coffee, but Petch (9) points out that the same fungus had been named *Corticium salmonicolor* by Berkeley and Broome many years before from material obtained in Ceylon, hence the latter name has the right of priority.

In 1897 Ridley (11) reported the presence of a disease of coffee in Selangor caused by a fungus with pink spore masses, which was named

Necator decretus by Massee (8), and in 1901 Zimmermann (17) reported the same fungus on coffee in Java and pointed out that it was generally associated with *Corticium javanicum*, not only on coffee but also on tea and other hosts.

Between 1904 and 1909 the fungus was reported under different names on rubber in North Borneo (12), Malaya (6, 13, 14), Ceylon (9), and Southern India (1). More recently it has been found on rubber in Sumatra (10) and Burmah (4). *Corticium salmonicolor* has been found on other hosts in the Cameroons (5) and in the Caucasus (15). In the West Indies a pink fungus on cacao has been referred to *Corticium lilacofuscum*, which may possibly be identical with *Corticium salmonicolor*.

In 1912 Rant (10) published an account of an investigation of *Corticium salmonicolor* with special reference to cinchona, the most important result of which was to establish the identity of the *Corticium* and *Necator decretus*, these being shown to be two stages of one and the same fungus.

Corticium salmonicolor is an omnivorous fungus and Rant mentions that it has been found on no less than 141 species of plants belonging to 104 genera and many different families. The disease has been found on Gymnosperms as well as on Dicotyledons, but no record has yet been made of it on Monocotyledons. Rant (10) states he has seen the fungus growing on the epiphytic fern *Drymoglossum heterophyllum* without apparently causing harm.

In Malaya, *Corticium salmonicolor* has been found recently on Para rubber, cocoa, *Coffea robusta, Gardenia sp., Hibiscus sp.*, camphor (*Cinnamomum camphora*), *Cassia sp.*, horse mango (*Mangifera foetida*), *Lansium domesticum*, lime, durian (*Durio zibethinus*), jak (*Artocarpus integrifolia*), *Averrhoa sp.*, mango (*Mangifera indica*), *Tephrosia Hookeriana*, *Indigofera arrecta*, and *Clithoria cajanifolia*. The number of fruit trees in this list is noteworthy, but the fungus is not often found on them.

In other tropical countries the fungus is of economic importance on other plants besides rubber. In Java, coffee and cinchona are seriously affected by it and in Ceylon the fungus causes a serious disease of tea.

Corticium salmonicolor is probably native in most of the countries in which it has been recorded. Many of the plants mentioned by Rant (10) as having been affected by it are native to Java and some of the plants on which the fungus has been found in Malaya are also indigenous. Anstead (1) states that the fungus is present on jungle

trees in the neighbourhood of rubber estates in Southern India. The fungus has probably spread from native hosts to plants that have been introduced, such as rubber, coffee, tea, and cinchona. There is one doubtful record of it on a jungle tree in Malaya, but one cannot say at present whether it occurs to any appreciable extent in the forests. Unfortunately *Corticium salmonicolor* has shown a considerable liking for rubber trees, and as far as Malaya is concerned *Hevea brasiliensis* is by far the commonest host for the disease. The disease is much more prevalent in some parts of the country than in others and there are some large areas where the disease has not yet been found. The disease is most abundant at present in the districts of heaviest rainfall and where large tracts of jungle remain.

Field Observations.

Pink Disease attacks rubber trees of various ages though it is not often seen on trees less than two years old. An attack often begins in a fork of a tree on account of the accumulation of water there, but sometimes the disease affects a branch in the middle and it has been seen occasionally to attack the main stem. The disease develops most rapidly during periods of heavy rain. In dry weather obvious signs of the fungus frequently disappear to appear again when the rains come.

The manifestations of Pink Disease on rubber trees are extremely variable. The disease is so called because the fungus often causes a pink incrustation on the branches or main stem, which is more specially developed on the under or shady side (shown on the right-hand side of Plate XVI, fig. 2). In this condition the disease is very striking and cannot be mistaken. The incrustation cracks irregularly after a time and the bright pink colour rapidly fades to a dingy white. There are, however, at least three other forms in which the fungus appears on rubber trees:

(*a*) Pink Disease frequently assumes the form of white or pale pink pustules arranged more or less in lines parallel with the branches. This is the "Höckerchen" form of Rant (10).

(*b*) At other times part of the fungus on the exterior consists of white or pale pink strands of a cobweb-like texture which run irregularly downwards over the surface, the strands being sometimes so delicate as to be overlooked (cf. Plate XVI, fig. 1). This is the "Spinnegewebe" form of Rant (10) and is usually the first stage to appear on an affected tree.

Fig. 1. Photograph of the cobweb-like form of mycelium of *Corticium salmonicolor* on a branch of a rubber tree.

Fig. 2. Photograph of the incrusting form of *Corticium salmonicolor* (to right) and of pustules of the *Necator* stage (to left) on a branch of a rubber tree.

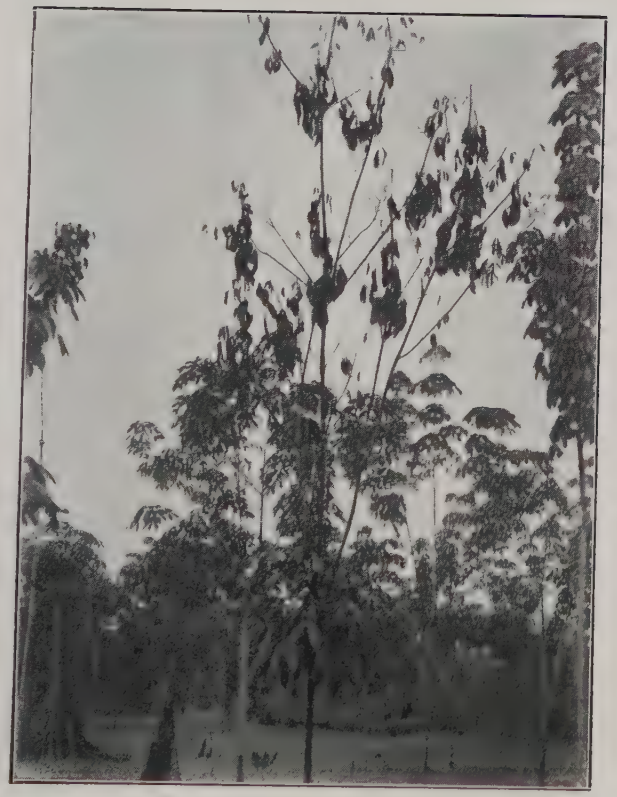

Fig. 3. Photograph of young rubber tree the upper part of which has been killed by *Corticium salmonicolor*.

Fig. 4. Photograph of rubber tree bearing "cankers" caused by *Corticium salmonicolor*.

(*c*) Finally there is the *Necator* stage which was formerly looked upon as a separate fungus, *Necator decretus*, Mass., but is now known to be a stage in the life-history of Pink Disease. Pustules of *Necator* are seen on the left-hand side of Plate XVI, fig. 2. The fungus in this condition consists of orange-red (not pink) pustules about one-eighth inch in diameter, each pustule being a mass of spores which serve to propagate the disease. In our experience the *Necator* stage has been confined to the side of the branch which is exposed to the brighter light. The *Necator* stage has always been found by us to be associated with other forms of Pink Disease, and so intimate is the connection between it and the other forms that it is difficult to understand the doubt that formerly existed as to the identity of *Necator decretus* and *Corticium salmonicolor*.

Spores of the fungus germinate on healthy bark especially where there is an accumulation of moisture. The mycelium which develops is entirely superficial at first, but after a time it penetrates the bark. When the mycelium reaches the laticiferous tissues, exudation of latex frequently begins and this runs down the bark and becomes blackened as time goes on. The weeping of latex from branches is often an indication of the presence of Pink Disease when from ground level no other sign of the disease can be seen. Once the mycelium has penetrated the bark it spreads upwards and downwards over and through the bark causing it to rot. The mycelium spreads more rapidly over the bark than through it. The fungus sometimes advances into the wood, this happening more frequently in small branches than in large ones. If the fungus spreads in the wood, the water supply becomes checked and the foliage of the affected branch turns brown and dies. On some undulating estates planters discover those trees which are affected by Pink Disease by observing from a hill the branches which are affected in this manner. Further information concerning the presence and development of the fungus in the wood is given below. Occasionally the fungus spreads downwards so vigorously that the whole of the upper part of the tree dies. In such a case as that represented in Plate XVII, fig. 3, the lower part of the tree sometimes makes an effort to recover by putting out new branches. When large branches are attacked by *Corticium salmonicolor* the progress of the fungus in the bark may be checked by a spell of dry weather and in this case an open, canker-like wound such as is shown in Plate XVII, fig. 4 is often formed as described by Petch (9). The formation of a callus on the margin of the wound tends to repair the injury and occasionally the disease is entirely

thrown off, but the fungus sometimes develops again over the bark which began to close the wound. Where the cankered areas have entirely thrown off the disease, the region around them is frequently blackened on account of oxidation of the rubber exuded when the disease was active.

Investigation of Hevea wood affected by Corticium salmonicolor.

Before the appearance of Rant's paper (10), Petch (9) stated that *Corticium salmonicolor* does not enter the wood to any appreciable extent. Rant noticed in cinchona that the fungus invaded the wood and the pith.

Branches of *Hevea brasiliensis* attacked by Pink Disease die in a manner characteristic of those attacked by a fungus which grows vigorously in the wood. Young branches are more often affected in this manner than are old ones. The rapid death of the leaves in such branches is a sure sign that the water supply is checked: this restriction is probably due to the activities of the fungus in the wood. It soon became clear that the fungus entered the wood of such branches, so a detailed investigation of the manner in which it invaded these tissues was made. Wood affected by *Corticium salmonicolor* is only slightly discoloured and differs greatly in this respect from wood permeated by *Diplodia cacaoicola*.

During the early part of 1914 a large branch of a rubber tree attacked by Pink Disease was obtained from an estate in Negri Sembilan in which the transition between healthy and diseased wood could be clearly traced. The branch was covered with the pink incrustation which ran out below into the cobweb-like form of the fungus. The cortical layers were dead and could be easily stripped off exposing the wood beneath.

When split longitudinally, the wood was seen to be sound for about two feet from the top of the branch, but the part below was dry and obviously diseased, except for a length of about nine inches which marked the transition between the healthy and diseased wood. This transition region had a moist, almost transparent, appearance and gradually passed below into the dry, diseased wood and above into the moist, healthy wood.

Diplodia cacaoicola, P. Hennings, is a common wound parasite of *Hevea brasiliensis* and has been found in some branches attacked by Pink Disease. A careful search for *Diplodia cacaoicola* was therefore made before investigating the mode of attack of *Corticium salmonicolor*. No external sign of *Diplodia* was observed and microscopical examination

failed to show any of the characteristic dark coloured hyphae of this fungus running through the vessels even in the most badly attacked portions of the wood.

Though *Diplodia cacaoicola* was absent, the wood was permeated with hyaline hyphae. A section of the branch including the transition area and a portion of the dead wood was taken and cut into numbered blocks throughout its length. Razor sections of the wood at different levels were made, the lower portion of the transition area being found most favourable for examination. Transverse sections through this part show the hyphae ramifying through the elements of the wood, being especially prominent in the vessels (Fig. 1). The wood of *Hevea*

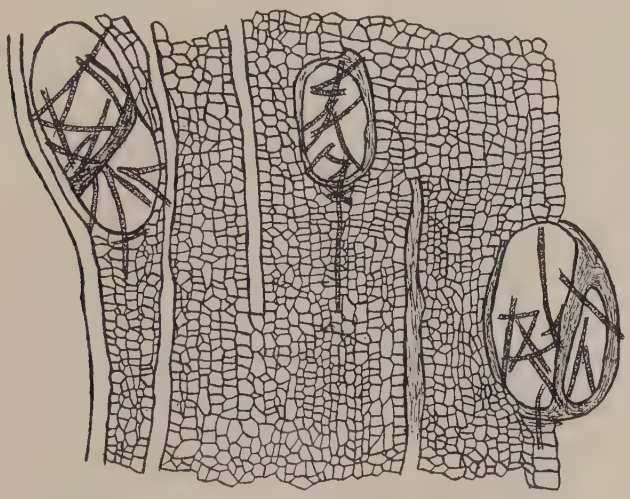

Fig. 1. Transverse section of wood showing hyphae in the vessels. ×40.

brasiliensis is mostly composed of fibrous elements together with a comparatively small number of large vessels and narrow medullary rays.

A study of longitudinal sections, both radial and tangential, shows the nature of the attack upon the wood. A favourable radial section shows the hyphae passing transversely through the wood along the medullary rays by way of which also the fungus passes from the bark into the wood. The fungus obtains food from materials stored in the ray cells which become filled with septate hyphae (Fig. 2). In radial section the medullary rays appear to be broad bands of infected tissue passing through the wood. At places where the vessels meet the

medullary rays, the mycelium travelling in the cells of the rays spreads out and enters the vessels (Fig. 3). All the elements of the wood become permeated with the hyphae which pass readily through the large pits without constriction. The deep medullary rays favour a quick passage transversely while the large pits allow a ready passage for the hyphae among the elements of the wood.

The most characteristic feature in the wood of *Hevea brasiliensis* attacked by Pink Disease is the presence of tyloses in the vessels (Figs. 4 and 5). Every specimen examined showed these bladder-like ingrowths from the living cells bordering the vessels, plugging up

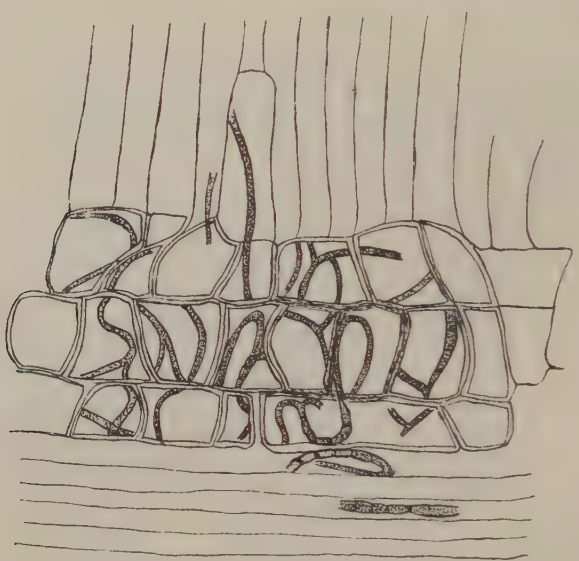

Fig. 2. Section showing hyphae in medullary ray cells. ×160.

the water courses. Specimens of healthy wood, of wood taken below the tapping area, and of wood attacked by *Diplodia cacaoicola* were examined, but in no case was there any indication of tyloses. Thus the formation of tyloses in *Hevea brasiliensis* appears to be a response to the attacks of *Corticium salmonicolor*.

The tyloses are of two types, (*a*) those in which the cells retain their thin cellulose walls (Fig. 4), (*b*) those in which the walls become lignified (Fig. 5). Both types are found in the same branch in adjoining vessels though the second type is rarely produced. The tyloses which become lignified lose their protoplasm and appear like

a number of small vessels included in a larger one. In the majority of cases only the thin-walled type occurs. Their contents are devoid of food reserves.

The response made by living cells to an injury usually results in an abnormal growth of neighbouring cells. In *Hevea brasiliensis* attacked by Pink Disease an abnormal bladder-like ingrowth of the

Fig. 3. Longitudinal section showing hyphae passing from medullary rays into vessels. Hyphae pass through without constriction. (Drawing somewhat diagrammatic.) × 160.

living cells bordering the longitudinal path of the fungus, *i.e.* the vessels, takes place.

The formation of tyloses as a traumatic response is presumably an attempt on the part of the host to check the passage of the fungus through the tissues. In this case it is obviously unsuccessful as the hyphae easily pass through the tyloses (Fig. 4). Failing this function,

however, they hasten the death of the branch by preventing the ascent of water.

This investigation of diseased branches explains the symptoms often observed when branches of *Hevea brasiliensis* are affected by Pink Disease. The fungus attacks the wood, vigorously pursuing its course up and down the stem through the vessels. Other vessels are blocked with tyloses for considerable distances while others again are filled with a brown gummy substance. Thus a large proportion of the functional

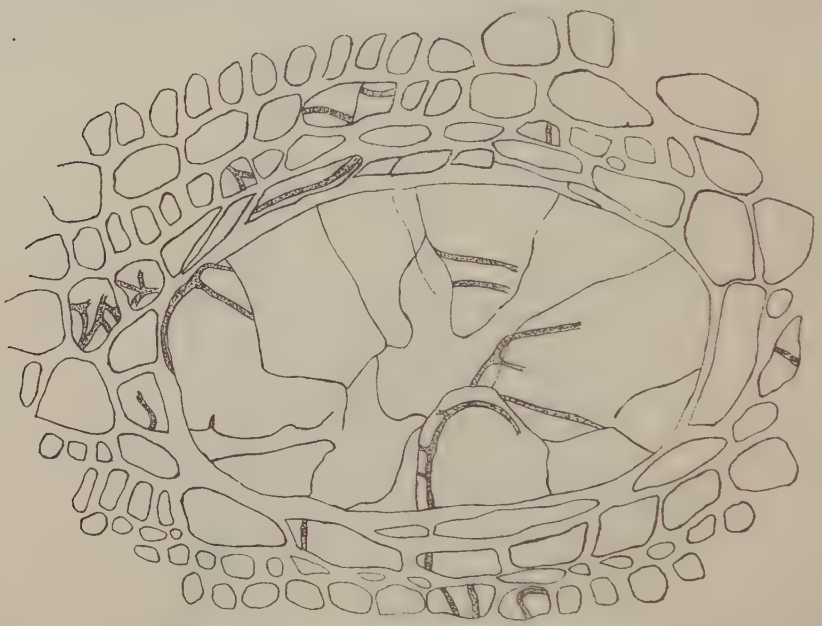

Fig. 4. Transverse section of vessel with thin-walled tyloses, showing hyphae passing through tylose cells. × 160.

vessels are rendered useless. This results in a serious diminution in the amount of water ascending to the leaves which droop and ultimately die.

Description of the Fructifications of the Fungus.

(*a*) *Basidial stage.* Zimmermann is responsible for placing the fungus in the genus *Corticium* and one gathers from his description and the accounts of subsequent writers that the pink incrustation is the basidial fructification. We found, however, that this stage was sterile in more than 80 per cent. of the large number of cases examined at

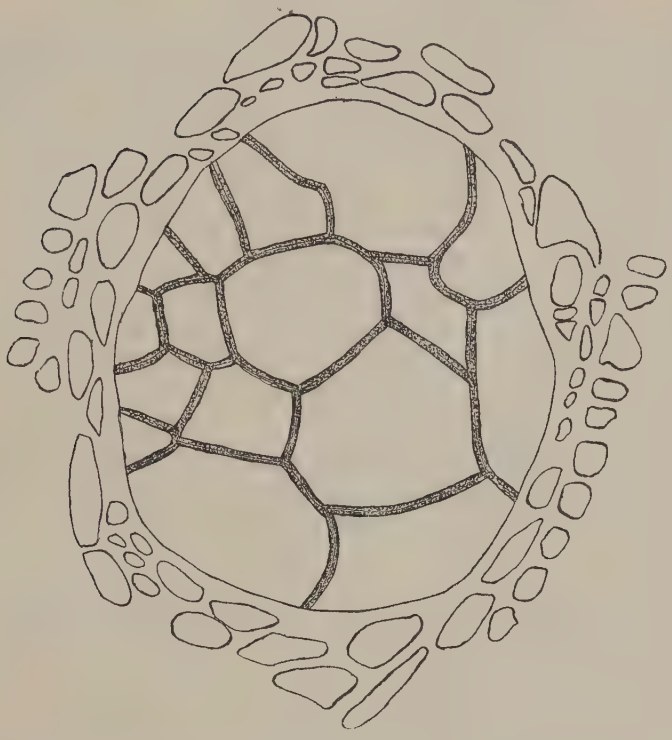

Fig. 5. Transverse section showing vessel with lignified tyloses. ×160.

Fig. 6. Section of pink incrustation with basidia. Section 6μ thick and stained with Coton Bleu and Orange G. ×400.

different times of the year. Great difficulty was experienced in finding basidia at all and it was not until after much searching that we recognised the type of incrustation that produced basidiospores. This form is thicker, has a more homogeneous surface, and when dry cracks into larger pieces than the sterile incrustation. It is remarkable that neither Zimmermann nor any other writer has called attention to the fact that the pink incrustation is frequently sterile.

One concludes from Zimmermann's description and figures that a typical hymenium is developed, but according to our experience the basidia are scattered and are irregularly arranged as in Fig. 6. The size of the spores is as given by Zimmermann and the sterigmata are noticeably long. We have not seen the basidia arranged even approximately as they are in Zimmermann's figure. The irregular distribution of basidia reminds one rather of an *Hypochnus* than a *Corticium*. Rant (10) has apparently made no special study of the basidial stage as he copies Zimmermann's figure of the hymenium and agrees with his description of it.

In North America, Stevens and Hall (16) have described a disease of pomaceous fruit trees caused by *Hypochnus ochroleucus*, Noack, which spreads over branches and twigs by means of mycelial strands and kills the leaves by enveloping them. The basidia are scattered and are irregular in form. *Corticium salmonicolor* seems to be more closely related to *Hypochnus ochroleucus* than to other species of *Corticium*. Bernard (3) also has described a disease of tea in Java which he attributes to a fungus named by him *Hypochnus theae*. Though there appear to be certain minor differences between this fungus and *Corticium salmonicolor*, the resemblances in the arrangement of the basidia and the character of the sterigmata and spores are very striking.

(*b*) *Necator stage*. The genus *Necator* was founded by Massee (8) in 1897 for the reception of a single species, *Necator decretus*, which was the cause of a stem disease of coffee in Malaya. It is now known that this is one of the stages of *Corticium salmonicolor*. The *Necator* stage consists of orange-red masses of spores, the individual spores being irregular in shape (Fig. 7) and hyaline when seen under the microscope. Each spore mass is waxy in consistency and it is likely that the spores become separated from one another only in wet weather when they are washed apart.

The mode of formation of these pustules is different from what one would gather by examining Zimmermann's figures, which have also been reproduced in Rant's paper (10). Zimmermann's figures

indicate an origin somewhat similar to that of a pycnidium, but we find that the mycelium aggregates beneath the outermost layer of cells of the branch, forming a kind of stroma which by growth ruptures the

Fig. 7. Group of *Necator* spores, teased out, showing irregular size of spores. ×400.

tissues of the host (Figs. 8, 9). The whole of this stromatic mass becomes converted into spores by the separation of the cells one from the other. The irregularity in the size and shape of the spores (Fig. 7) is due to this peculiar method of spore formation. The dimensions of the

Figs. 8. Section showing early stage in development of a *Necator* pustule: Stroma forming under the outer layer of cells. ×160.

spores are 14–20 μ × 8–10 μ. In other species of *Corticium* and *Hypochnus* small sclerotia about the size of pustules of *Necator* are produced and in *Corticium salmonicolor* such sclerotial aggregates may have become modified into spore masses by separation of the constituent cells instead of forming resting bodies.

Necator spores germinate readily in distilled water and in nutritive solutions (Fig. 10).

Fig. 9. Section of fully developed *Necator* pustule. ×400.

In Malaya the *Necator* has been found much more frequently than the basidial stage and it is likely that it takes the more active part in the dissemination of the disease.

The other forms of *Corticium salmonicolor* are constantly sterile.

Fig. 10. Germination of *Necator* spores, after 12 hours in damp chamber. ×400.

The fungus is probably chiefly spread by wind, though it is possible that it is also disseminated by red ants and other insects which visit rubber trees. It is possible too that not only spores but also small portions of the sterile incrustation are disseminated by these means.

The incrustation retains its vitality for a considerable time and, as stated above, it cracks into small pieces as it gets older, and these, breaking away, may be carried to other trees in one or other of the ways mentioned.

A species of *Nectria* is often associated with cases of Pink Disease of long standing, but as far as is known at present it is purely saprophytic and develops only after the bark has been killed by *Corticium*.

Pure Cultures of Corticium salmonicolor.

At the beginning of these investigations efforts to obtain pure cultures of the fungus were unsuccessful. We soon recognised that the incrusting form was usually sterile so we gave up attempts to obtain a deposit of basidiospores with which to start pure cultures. Failing basidiospores, small pieces of the pink incrustation were cut out with a sterile knife and placed on slants of salep agar. The fungus quickly developed, but the cultures were usually contaminated. Subcultures started from these were eventually obtained which were probably pure. No further attempt to obtain pure cultures in this way was made because at this stage one of us obtained material in the field which appeared somewhat unusual at the time. This material bearing orange-red pustules was sent to the laboratory and immediately examined, when the pustules were seen to consist of masses of spores, these being the *Necator* form of the fungus which was subsequently obtained frequently.

Pure cultures were obtained from the *Necator* spores. These were teased out on sterile glass slides; some were placed in damp chambers on salep agar, others directly on test tube slants of the same medium. The spores quickly germinated in the damp chambers (Fig. 10); these were kept under observation for three or four days in order to see whether the cultures remained pure. After this, transfers from the damp chambers to test tube slants were made. As these cultures and those obtained by placing spores on test tube slants direct were identical, little doubt remained as to their purity. The mycelium was pure white and did not grow copiously. After a period of about ten days a faint pink colouration appeared in the cultures. The agar cultures were kept for several weeks, but no further development took place. Other cultures were then made by transferring small portions of the mycelium to blocks of sterilised *Hevea* wood placed in tubes (a figure and explanation of the form of tube used is given in Fig. 11). About 50 per cent.

of the attempts to start cultures from small pieces of mycelium were unsuccessful.

The cultures on wood blocks obtained in the first place from *Necator* spores develop quickly. The mycelium grows profusely and remains white for seven to ten days. It spreads over the block and into the

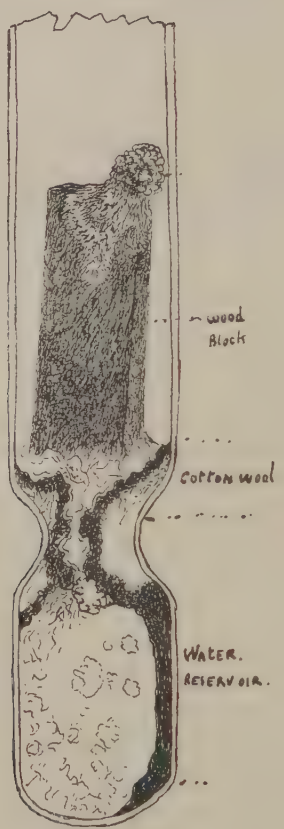

Fig. 11. Culture tube: wood block covered with mycelium which has grown into water reservoir covering the water with a film of hyphae on which *Necator*-like masses have formed. Large *Necator*-like masses at top of wood block.

cotton wool placed at the base of the tube. Growing in the cotton wool the mycelium begins to turn a pale pink colour which gradually spreads over the whole culture. Subsequently the mycelium in the cotton wool becomes a bright rose colour. The mycelium passes from

the cotton wool into the reservoir of water where it becomes aggregated.

The cultures started originally from the pink incrustation were now used to inoculate sterilised blocks of *Hevea* wood. The cultures so obtained were identical with those derived from the *Necator* spores so that, apart from other evidence, this alone indicated the connection between the two forms.

The cultures on wood blocks were placed under different conditions to ascertain whether these influenced the development of the colouration. Some were placed in the laboratory in diffuse light, others in direct sunlight, and others in darkness. Rant (10) states that his agar cultures placed in darkness remained white though after illumination for a short time a pink colouration appeared. In our experiments the cultures grown in darkness behaved like those in the light except that the pink colour did not develop so rapidly.

In older cultures the mycelium often turns a dirty brown colour as though some impurity had entered. However, small portions of this mycelium seen by transmitted light show the characteristic colouration. The hyphae in the old brown cultures are closely aggregated, corrugated, septate, much vacuolate, with numerous clamp connections. The hyphae in young cultures are septate, vacuolate, but not corrugated, whilst clamp connections are less numerous.

In one culture placed in bright light and in another kept in diffuse light an aggregation of hyphae took place along the upper edge of the blocks to form a bright pink mass. The aggregation continued until a solid mass half an inch high and half an inch in diameter was formed, attached to the block by a thinner base. It resembled a number of closely attached *Necator* pustules (Fig. 11). Examined microscopically the mass was found to consist of short cells somewhat irregular in size forming a kind of pseudo-stroma.

These *Necator*-like masses are often formed in the cotton wool at the base of the culture tubes and sometimes upon the surface film of mycelium in the reservoir. Their spore-like nature is indicated when small pieces are used to start new cultures upon wood blocks. In every case the mycelium develops copiously within 24 hours, while, as stated above, 50 per cent. of attempts to start new cultures with portions of the usual form of mycelium result in failure. Under natural conditions it is probable that the cells forming these pustular masses would become detached from one another in a manner similar to those forming typical *Necator* pustules.

Rant (10) states that he did not obtain either fruit form in his cultures, but calls attention to the formation of "paraplectenchymatische mycelienknäuel," which he obtained now and again in his cultures and which had been previously observed by Koorders (7) in cultures of *Necator*. The *Necator*-like masses obtained by us probably correspond to these structures observed by Rant and Koorders. Rant confined his attention chiefly to agar cultures, but in our cultures on this medium we did not obtain anything approaching a fruiting form, though our efforts in this direction were not long continued after we found wood blocks so favourable for culture work.

Some of the wood blocks on which cultures had been grown were sectioned. It was seen that the fungus had spread to the centre of the block and that the mycelium had penetrated the elements of the wood in the same manner as in wood naturally infected. Tyloses were absent.

Inoculation experiments.

Rant's inoculation experiments (10) experimentally demonstrated the connection between *Corticium salmonicolor* and *Necator decretus*. Our inoculation experiments to be now described were carried out to test the conditions which favour or hinder the development of the fungus on *Hevea brasiliensis*. Correlated experiments upon other hosts were kept under observation at the same time.

The first series of inoculation experiments was carried out on rubber trees three-and-a-half years of age. The inoculations were made on the 4th of February, 1914, with pieces of the pink incrustation obtained from a diseased rubber tree. The inoculations were covered with cotton wool pads which were moistened every morning for the first three weeks as the weather was dry during this period. The pads were kept in place by rubber bands.

Little rain fell between the date of inoculation and March 10th, but between March 10th and March 16th there were daily showers. The fungus appeared during this wet spell, 13 out of 29 inoculations being successful. Of this number 8 out of 13 (60 per cent.) were obtained upon uninjured parts; 5 out of 16 (30 per cent.) upon wounded surfaces. These results provide further evidence that *Corticium salmonicolor* acts rather as a vigorous parasite on uninjured parts than as a wound parasite.

Between March 16th and April 14th the weather was dry and no rain fell for considerable periods. On the latter date only a few trees

showed any trace of the fungus and it appeared that the fungus was dying or was hibernating in the bark.

The inoculations were examined from time to time, but the fungus made no further progress. This result may be attributed to the dry weather which followed the moist spell ending on March 16th. A similar result is often observed on estates, for branches of rubber trees may recover from an attack of Pink Disease if a long spell of dry weather intervenes.

Similar experiments were carried out in the field at the same time on *Coffea liberica*, *Cinchona succirubra*, *Cinchona ledgeriana*, and *Cinnamomum camphora*, six inoculations being made in each host, three being in wounds and three being on unwounded surfaces. Only one successful inoculation was obtained and that on *Coffea liberica* where the cobweb-like form of mycelium appeared over the wound in which the fungus had been inserted.

The results indicate that these hosts are attacked less vigorously than is *Hevea brasiliensis*. These experiments indicate the possibility that small portions of the sterile incrustation may disseminate the disease. These easily break away and are blown about by the wind. Under favourable conditions the mycelium may develop and give rise to a new infection.

Inoculations were subsequently commenced in the laboratory with pure cultures of the fungus. Rubber seedlings and plants of *Gardenia sp.* and *Cinchona succirubra* were inoculated either by tying wood blocks used in growing pure cultures upon the stem, or by placing pieces of the mixture of mycelium and cotton wool from the base of tubes in wounds in the stem or in contact with the stem. Six inoculations of rubber seedlings and of *Cinchona* and three inoculations of *Gardenia* were made on July 10th, but by August 19th no success had been obtained. In these experiments the plants were kept under bell jars. The inoculations on *Gardenia* and *Cinchona* were overgrown with moulds within a week. In several inoculated rubber seedlings, however, the fungus appeared to grow strongly at first, but it soon weakened, and on August 19th the host plants were quite healthy. The experiments indicate that Pink Disease is not likely to attack very young plants of Para rubber.

Inoculations of rubber trees with pure cultures were also carried out in the field, trees standing in an overgrown nursery being used for this purpose. Fairly large branches of the outside trees were inoculated on July 14th with the mixture of mycelium and cotton wool from the

base of the culture tubes. Similar experiments were performed at the same time with *Coffea liberica* as host. The following table summarises the results on August 19th:

Name of host	Type of inoculation	No. of inoculations	No. of successful inoculations
Hevea brasiliensis	placed in contact	12	1 cobweb-like form
	placed in wounds	12	1 cobweb-like form
Coffea liberica	placed in contact	4	1 cobweb-like form
	placed in wounds	4	——

Thus successful inoculations of both rubber and coffee were obtained with pure culture material of the fungus.

Rant experimentally demonstrated that *Corticium salmonicolor* does not exhibit the phenomenon of specialised parasitism. Thus this fungus occurring on one host is not limited in infective power to that particular host or a few others, but can attack a wide circle of plants. It passes readily under favourable conditions from one host to another. Only in very wet weather does the fungus spread rapidly, as was obvious in our field experiments. Relatively few of our inoculations were successful and this we attribute to the difficulty of keeping them moist during the spells of dry weather which intervened during the progress of the experiments.

Treatment.

It must be pointed out in the first place that, exceptional cases apart, spraying with fungicides is impracticable and to some extent also useless. Spraying a rubber plantation with trees 30 to 60 feet high is an entirely different proposition from spraying an orchard containing trees only 20 feet or so high. There would have to be something in the nature of a revolution in spraying methods to enable a mature rubber plantation to be sprayed effectively so as to check Pink Disease. Spraying experiments which we recently carried out with two modern machines provided with extension rods show that the maximum height for which they can be used under estate conditions is 25 to 30 feet, and this height is only obtained with difficulty by coolie labour. Longer extension rods have been tried, but the difficulties of manipulation under estate conditions are so great that they cannot be used with

success for spraying large numbers of trees. Hence if a mature rubber plantation were sprayed for Pink Disease the upper branches of the trees would remain unprotected and these are the parts most liable to the disease. Again, in view of the regularity of the rainfall in the Federated Malay States a single spraying would be useless. To be effective at all in such a climate, spraying would have to be repeated at frequent intervals.

Spraying with Bordeaux Mixture or Lime Sulphur, preferably the former, might be effective in checking the disease in plantations not more than three years of age if there was danger of it breaking out in epidemic form, but fortunately there is yet no indication of this in Malaya. In Southern India where there is a prolonged dry season, Anstead (2) reports that painting the forks of young trees with Bordeaux Mixture before the coming of the monsoon reduced the percentage of trees affected from 1·34 to ·56, ·07 (three applications of the fungicide were given here), and ·7 per cent. in various cases. In our opinion estates infected to the extent of about one per cent. would be preferably treated by cutting out or by tarring as described below. Another circumstance, which would only occur exceptionally, and in which spraying a limited number of trees might be undertaken, is where the disease is confined to one portion of an estate. In conjunction with treatment within the infected area in the manner described below, it might be advisable to spray carefully a belt of trees around this area as a precautionary measure.

When Pink Disease first appears in a rubber plantation it is usually distributed in a sporadic manner. It is of the utmost importance that the disease should be dealt with vigorously from the outset by cutting off and burning the affected parts. In most plantations where Pink Disease appears for the first time only a few trees are attacked. In such cases diseased branches should be cut off at least two feet below the lowest point where there are obvious signs of the fungus and it is preferable to cut them off flush with the main stem or larger branch.

Where a large number of trees are affected on an estate the manager will probably hesitate before he cuts out the disease in this drastic manner. As an alternative, branches and main stems which appear to have a chance of recovery should be covered with tar for two feet above and below the region over which the fungus is evident. If the disease is dealt with in this way in the early stages many branches and sometimes entire trees may be saved. Even when the fungus has penetrated the bark to a slight extent the external application of tar

appears to check its progress. It has been urged that the diseased bark should be removed before tar is applied or even that tar should first be placed over the affected parts, the rotten bark removed, and then tar subsequently applied again. These are excellent ideals and if expense were no object would be strongly recommended. Experience has shown, however, that where tar is applied thoroughly without previous removal of diseased bark good results are obtained as long as the treatment is renewed within a month if necessary. Trees treated with tar for Pink Disease should be examined within a month, and if the fungus has spread, tarring should be tried again. If two applications of tar are found useless in checking the disease the affected parts should be cut out and burnt. On several estates where this mode of treatment has been adopted, Pink Disease has been reduced to a minimum. If tar is used to check Pink Disease it is essential that the work should be done under good supervision, for if done carelessly the money spent on it will be wasted. It is important also that diseased trees should be treated at an early stage. In certain cases, *e.g.* when the leaves of an affected branch have died, it is obviously hopeless to apply tar. The only thing to be done in such cases is to cut out and burn the diseased portions. The use of a concentrated Lime-Sulphur mixture has been tried instead of tar, but it is difficult to check the use of it and it is readily washed off by rain, hence it is not a good substitute and is not recommended.

Planters sometimes have difficulty in burning diseased branches on account of persistent rain. If it is impossible to burn the diseased parts directly, they should be drenched with a 10 per cent. solution of sulphate of copper, removed from the plantation and buried in the ground some distance away from the rubber trees. It must be remembered, however, that there is nothing so good as fire for the destruction of fungoid pests. In this connection mention may be made of the fact that another pink fungus, *Oospora sp.*, which is harmless, usually develops on wood in Malaya a few days after being burnt. This fungus has been several times mistaken for Pink Disease.

Where Pink Disease has appeared in an estate, a pest gang should be formed if not already established and the size of the gang should be such that it can go over the whole estate and treat diseased trees on the above lines once every three or four weeks. Pink Disease develops rapidly and any longer interval is too great to allow of it being dealt with effectively. Where a considerable amount of Pink Disease is present one can hardly expect to eradicate it completely so the expense

of maintaining a pest gang must be met. The rubber plantation industry is dependant on the health of the trees so it would be suicidal policy to grudge money for treating disease.

Any plants besides rubber which are affected by Pink Disease in the neighbourhood of estates should be destroyed as the fungus passes readily from one host to another. The manifestations of the disease on other plants are the same as on rubber.

If the measures indicated above are carried out, the disease should be kept under control, but any neglect of it will be dearly purchased. This disease of rubber trees is more common in Malaya at the present time than the die-back caused by *Diplodia* and it is a disease which will have to be watched carefully.

In conclusion we wish to express our thanks to Mr F. de la Mare Norris for kindly making the drawing from which Fig. 11 has been reproduced.

Summary.

1. The distribution, hosts, and mode of action of Pink Disease are described and its importance as a disease of plantation rubber is emphasised.

2. The various forms of *Corticium salmonicolor* are described. It is pointed out that the fungus is not a typical *Corticium* and that the pink incrustation is very frequently sterile.

3. *Corticium salmonicolor* often affects the wood as well as the bark of rubber trees. Its action on the wood is described in detail. The formation of tyloses appears to be a response to the presence of the fungus in the wood.

4. Pure cultures of *Corticium salmonicolor* have been established on salep agar and on *Hevea* wood.

5. Inoculation experiments both with natural material and with the fungus grown in pure culture have been successful.

6. *Treatment.* (*a*) Spraying is not recommended except in particular cases. (*b*) The disease is best dealt with either by cutting out infected branches, or by treating affected parts with tar. Detailed instructious are given as to the manner in which these operations should be carried out by a pest gang.

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AN ATTEMPT TO MEASURE THE LOCAL AND SEASONAL ABUNDANCE OF THE SWEDE MIDGE IN PARTS OF YORKSHIRE OVER THE YEARS 1912 TO 1914.

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(With Plate XVIII, 2 Text-figures, and 5 Charts.)

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I. Introduction.

The object of this paper is twofold, firstly to describe an attempt to measure the relative density in numbers of one particular insect pest at different times in certain selected places; secondly, to examine the extent to which the fluctuations in the figures collected can be accounted for.

It is, of course, notorious that the abundance of insect pests varies enormously in different years and in different localities, but so far as I know little has been done in the way of actually measuring such fluctuations in abundance. Unless such measures can be obtained there is, of course, no definite basis on which to correlate differences of abundance with differences of conditions. Various factors may be suggested as of importance in determining the degree of prosperity of an insect, but without a statistical basis it must necessarily be difficult or impossible to discuss such factors with precision.

The only way in which greater precision can be ensured appears to be to undertake a census over a period of years and preferably over an area which includes districts which differ amongst themselves in the conditions to which the insect is exposed in them. The conclusions which are drawn from such a census should then so far as possible be subjected to the test of experiment.

The present paper is an account of work carried out on those lines on the Swede Midge, *Contarinia* (*Diplosis*) *nasturtii*, Kieff. The country worked in was the area in the East Riding of Yorkshire shown on the accompanying charts, and also a small area round Garforth in the West Riding.

My object was to obtain a figure for each of the districts into which I divided my area, which should be roughly proportional to the number of Swede Midge individuals per acre of swedes. With many crops and many insect pests methods like those shortly to be described could not be used. In the case of the Swede Midge, however, a combination of fortunate circumstances makes it quite practicable to carry out a fairly accurate census, and to do so, if desired, two or three times in one summer. These particular circumstances are briefly as follows:

1. The number of swede plants in a row can easily be counted.
2. It is easy to recognise at a glance the characteristic distorted appearance of the swede plant produced by Swede Midge attack.
3. Signs of Swede Midge attack on a plant last, as a rule, about six weeks after the eggs have been laid.
4. Swede Midge maggots remain on the plant upon which they have been born until they have completed the feeding stage. They do not move from one plant to another.
5. So far as I am aware the Midge never kills a plant. However badly a plant may be attacked, it remains alive and shows, by its distorted appearance, that it has been attacked.

Fig. 1. Sound swede plant.
(From Mr Taylor's *Cabbage-Top in Swedes.*)

Fig. 2. Young swede plant, showing early stage of crumpled-leaf condition.

Fig. 3. Swede leaf, showing later stage of crumpled-leaf condition.

Fig. 4. Cowlam Well Dale. A typical narrow wold valley (District V). August, 1913.

6. The several broods of the Midge do not succeed each other too quickly or overlap too much for it to be impossible, by choosing the right time, to make a census for each brood in an area such as that in which I worked.

II. *Life-history of the Swede Midge.*

The life-history of the Swede Midge and the distortion which it causes to the swede plant are described by Mr T. H. Taylor in *Cabbage-Top in Swedes*[1]. The following short account is based on that in Mr Taylor's paper.

The flies are first found in swede fields in June. The eggs are laid in strings and clusters mostly near the base of the leaf-stalks, but some on the leaf-blades; the younger leaves are almost always the ones to be attacked. The eggs hatch in about four days and the maggots feed on the superficial tissues. The larval stage lasts about three weeks. When full grown the maggots go down into the ground and the flies come out, in the case of the summer broods in from two to three weeks. The normal number of broods for the season appears to be three, although in the hot summer of 1911 there were four broods. The winter is spent in the soil.

Mr Taylor describes and figures the distortion to the plant caused by the maggots. (Cf. figs. 2 and 3, Plate XVIII, of the present paper.) He says "In three or four days after the larvae have started to feed the plant begins to show signs of being damaged. The stalks of the affected leaves become swollen, and bending sharply inwards across the top of the plant, press upon and compact the terminal bud. Moreover, the leaf-blades, at those areas where the larvae are feeding, become delicately crumpled, thus resembling the leaves of a savoy cabbage." Mr Taylor also proved that the Swede Midge was one cause of what he calls "many-neck" in swedes, that condition in which the plant has several necks or main shoots instead of the single one of a normal plant.

My own observations agree with Mr Taylor's account. I think, however, that in 1913 the time elapsing between the maggot's entering the soil and the emergence of the fly was often as long as a month. It will be seen that in this paper I am concerned only with the crumpled-leaf condition, and not with "many-neck." Besides the Swede, *Brassica*

[1] Publications of the Yorkshire Council for Agricultural Education, No. 82.

rutabaga, Mr Taylor has found the following plants to be attacked by the Swede Midge:

Brassica napus	Turnip.
B. rapa	Rape.
B. oleracea	Cabbage.
B. sinapis	Charlock or Wild Mustard.
Raphanus sativus	Radish.
R. raphanistrum	Wild Radish.

Turnips I have found by observation to be attacked only to a very small extent. Rape occupies an area small compared with that of swede crops. Cabbage and Radish occupy a very small acreage compared with that of swedes. Wild Radish is not a very abundant weed, but Charlock is found in plenty.

Bezzi and Kertesz in their *Catalog. Palaearc. Dip.* give as host plants of the Midge,

Nasturtium palustre	Marsh Watercress.
N. silvestre	Creeping Watercress.

These plants are found in wet situations in Holderness, but whether they are attacked there by the Midge I cannot say.

The relation of the Midge to wild hosts is a question which I have not yet investigated. But on p. 94 I explain why I believe wild hosts are not of much importance in determining the abundance of the Midge in a district.

III. *Methods.*

Swedes are grown in rows which are from 24 to 28 inches apart, and in singling the plants are left about 12 inches apart. It is therefore perfectly easy to count the number of swedes in a row. It is also quite easy to tell at a glance which plants have been attacked. Moreover, the maggots spend the whole of the feeding period within a very short distance of the point where the eggs were laid. They do not move from one plant to another. And the distortion brought about by the Swede Midge can easily be distinguished from that caused by other insects. As Mr Taylor says (p. 12), the leaves "retain to the end discoloured traces of the injury, and since the crumpled character persists, it follows that the observer can readily recognize, by their leaves, swede plants that have been attacked by the Midge, although neither flies, eggs, nor larvae may be present." I did, of course, come across plants which I was not able to say with certainty had or had not been attacked, but

as I made all the observations myself there was not more than one personal equation as a source of error. And I took it as a guiding rule in estimating percentages of attacked plants, when the maggots had left the plants, in doubtful cases to count as attacked only those plants of which one or more leaves showed a scar as well as the supposed crumpled appearance. This would mean that a few slightly attacked plants were not counted as attacked, but it was as good a rule as I could find. I am confident therefore that the error due to failure to recognize attacked plants was a very small one. Thus by simply counting the number of attacked plants in a row the percentage in that row could be calculated.

I have found by a laboratory experiment that one female fly can lay enough eggs to bring about the characteristic crumpled leaf appearance. And after examining a number of attacked plants with eggs or maggots on them, I think that the average number of eggs laid on a plant may be put at about fifteen, that estimate being, I believe, low rather than high. I have estimated the number of eggs in a few midges cut into microtome sections to be about a hundred. This suggests that one midge may infect from one to, say, ten plants. Several times I have found either two or three attacked plants adjacent to each other in a row of swedes, separated from the next attacked plant in that row be several hundred plants. It is probable, therefore, that these two or three plants were selected by one and the same female for oviposition. The number of swede plants in an acre after singling is about 20,000. From these data, therefore, if it served any useful purpose, we could calculate the number of female flies which would be needed to infect any particular percentage of plants in that area.

I should point out that in the operation of singling, which is performed, as a rule, from four to six weeks after sowing, a large proportion of the plants are knocked up with the hoe, and die. In early sown fields singling may be finished before any or many of the flies have made their appearance. In later sown fields, on the other hand, some plants will be attacked before and some after singling, when the number of plants is greatly reduced. And sometimes in the latest sown fields singling may not even commence until all the flies of the first brood are dead. This must be one reason why, as will shortly be shown to be the case, the figures from late sown fields are lower than those from early sown fields, for all the figures were obtained after singling had taken place.

My usual method was to find the number of attacked plants in each

of two rows widely separated from each other in each field visited. If the figures from the two rows agreed fairly closely, I took the percentage of attacked plants calculated from those two rows as the percentage for the field. If there was a considerable difference between the two figures, I would examine one or more further rows. Sometimes in the Brood II or Brood III surveys, when it was necessary to hurry through the survey as quickly as possible, I would examine only a single row. This I took care was about half way between the two rows examined in the first survey. I also found out whether each field was sown in May or June by asking people I met in the fields, or failing them I would estimate by eye, a thing which I was able to do with very fair accuracy. In this way a figure for the percentage of attacked plants and the approximate sowing date for each field visited were obtained. Each year I examined about 200 fields, examining the same fields in all the surveys of any one year.

I should point out that as the leaves grow from the heart of the plant to the outside and finally fall off, signs of attack do not last for more than about five or six weeks.

In 1913 in the East Riding the three broods of that summer did not, so far as I was able to tell, overlap. In 1914 they did overlap. I call the second survey the Brood II survey, and the third the Brood III survey, because I am sure that flies of those particular broods were respectively responsible for the greater part of the attack in each case. But it is not impossible that in the second survey some plants were counted which had actually been infected by flies of the third brood, and that in the third survey some plants were counted which had been infected by flies of a fourth generation. The two later surveys which were hurried through quickly are therefore surveys of the numbers of plants which could be seen to have been attacked at the stated times at which the surveys were carried out. The dates of the surveys and the tests applied to their accuracy are given in the Appendix. These tests show, I consider, that the figures obtained in my five surveys were sufficiently accurate for the purposes to which I put them. After the conclusion of the last survey charts were constructed, one for each year, on which all the fields examined were marked. The East Riding area was then divided into eleven districts and Garforth made a twelfth. The ground occupied by the town of Bridlington is not included in any district. All small plots near to swede fields of the previous year were omitted from calculations for a reason which will appear shortly.

For each survey in the different districts a figure was calculated which may be taken as a measure of the relative numbers of the Midge per acre of swedes, and may be called the **Swede Midge index**. This figure was obtained in the following way. For each survey the average percentage of attacked plants in the fields examined in each district was calculated. In speaking of the percentage of attacked plants in a field the abbreviation "% CL" is often used, meaning the percentage of plants showing the crumpled-leaf condition. An average figure is therefore sometimes referred to as the "average % CL." When a calculated average % CL for a district was between two whole numbers, the higher whole number of the two was taken as the index. For example, whether the figure was 7·1, 7·6, or 8·0, the index was taken as 8. The indices are tabulated in Tables 2 and 3 and plotted on Charts 1 to 5.

In my East Riding area there are two chief types of country. In the two Holderness districts the whole area lies below the 100 ft. contour and is quite flat. The boulder clay soil is deep and strong and requires artificial drainage. The Wold part of the area is higher, though nowhere as high as 600 ft., and contains numberless valleys (see fig. 4, Plate XVIII). A thin, comparatively light soil covers the porous chalk, and artificial drainage is unnecessary.

From figures published by the Board of Agriculture[1] I calculate that the proportion of land under turnips and swedes in the East Riding is about one acre in ten. This proportion is higher than in any other county in Great Britain. Swedes are probably grown on about one acre in twenty.

IV. *Statement of results.*

The more striking features of the appended tables may be briefly summarized.

1. In May sown fields the attack of the Midge is greater than in June sown fields.

2. The first brood of 1912 was a very large one. That of 1913 was a very small one. The progress of the Midge in the East Riding area from the first brood of 1913 to the third brood of 1914 is shown in a simple manner in the following table:

[1] Abstract of Agricultural Returns for Great Britain, collected 4th June, 1910.

		1913 Brood I	1913 Brood II	1914 Brood I	1914 Brood II	1914 Brood III
Number of districts with indices	1, 2, or 3	11	8	7	4	3
„	4, 5, or 6	—	3	4	7	2
„	7, 8, or 9	—	—	—	—	3
„	10 or more	—	—	—	—	3
Total number of districts		11	11	11	11	11

At Garforth the progress was more rapid. The indices were:

1913 Brood I	2
1913 Brood II	No survey
1914 Brood I	22
1914 Brood II	32
1914 Brood III	76

3. In the East Riding area the indices are on the whole higher in the northern part of the area than in the southern. If we divide the whole area into two parts, a northern part containing districts I, II, III, IV, V, and VI, and a southern part containing districts VII, VIII, IX, X, and XI, and calculate for each survey the average percentages of attacked plants in the fields of these two parts, we get the following figures:

	Northern part		Southern part	
Survey	Number of fields examined	Average % CL	Number of fields examined	Average % CL
1913 Brood I	75	1·3	76	0·8
1913 Brood II	75	2·7	76	2·8
1914 Brood I	80	3·1	109	1·6
1914 Brood II	80	4·3	109	2·4
1914 Brood III	80	9·7	109	4·0

4. The coast districts in the East Riding area have on the whole distinctly lower indices than the inland districts. On dividing the area into a coast region, including districts I, II, III, and X, and an inland region, including districts IV, V, VI, VII, VIII, IX, and XI, and finding the average percentage of attacked plants in the fields of these two regions, we get the following figures:

	Coast region		Inland region	
Survey	Number of fields examined	Average % CL	Number of fields examined	Average % CL
1913 Brood I	53	1·1	98	1·2
1913 Brood II	53	1·4	98	3·5
1914 Brood I	52	1·8	137	2·3
1914 Brood II	52	1·9	137	3·7
1914 Brood III	52	3·2	137	7·6

These differences in abundance of the Midge of the different broods and in different localities stand out clearly enough. In the following section I try to explain them.

V. *Explanation of results.*

(*a*) *Conditions determining the percentage of plants attacked by flies of Brood I of the Swede Midge in a given field.*

Apart from conditions affecting the whole of any particular Swede Midge District in any particular year the following conditions are important in determining the percentage of plants attacked by Brood I of the Midge in any given field.

(1) Date of sowing.

Early sown fields are attacked worse than late sown fields. This is shown in Tables 2 and 3. As already explained singling is doubtless in part responsible for the figures collected in June sown fields being lower than those from May sown fields. But this explanation will not account for the complete absence of attacked plants from many June sown fields, which I frequently found to be the case, for swede hoers are almost all unaware of the existence of the Midge. The Midge does not infect the plants until they get into rough leaf. The flies of the first brood appear in June, a few sometimes in July. Consequently early sown fields are exposed longer to attack than late sown fields.

(2) Distance from a swede field of the previous year, and

(3) Size of field.

In 1913 and 1914 I found that numerous small plots of early sown swedes next to swede fields of the previous year had percentages of infected plants high compared with the percentages in other fields in the same district. The following are examples of such 1913 fields:

	% CL	Highest other % CL in district
A. District IX	6	3
B. „	11	
C. „	30	
D. District VII	19	8
E. „	24	

The graph, Fig. 5, is of interest in this connection. In this graph the % CL is plotted for different rows, of which the numbers are given, in a 1913 swede field next to a 1912 swede field. The left side of the graph represents the side of the 1913 field near to the 1912 field, the right side the side of the field remote from that field. Similar graphs

could be drawn for other similarly situated 1913 fields and for 1914 fields near to 1913 fields. The graph shows a quick drop in % CL as the distance from the 1912 field increases, the first few rows being badly attacked, while the middle and far side of the field are not much, though a little, worse attacked than any part of a field not near to a 1912 field. Thus in a small field near to a swede field of the previous year, if both fields were early sown, the percentage of attacked plants

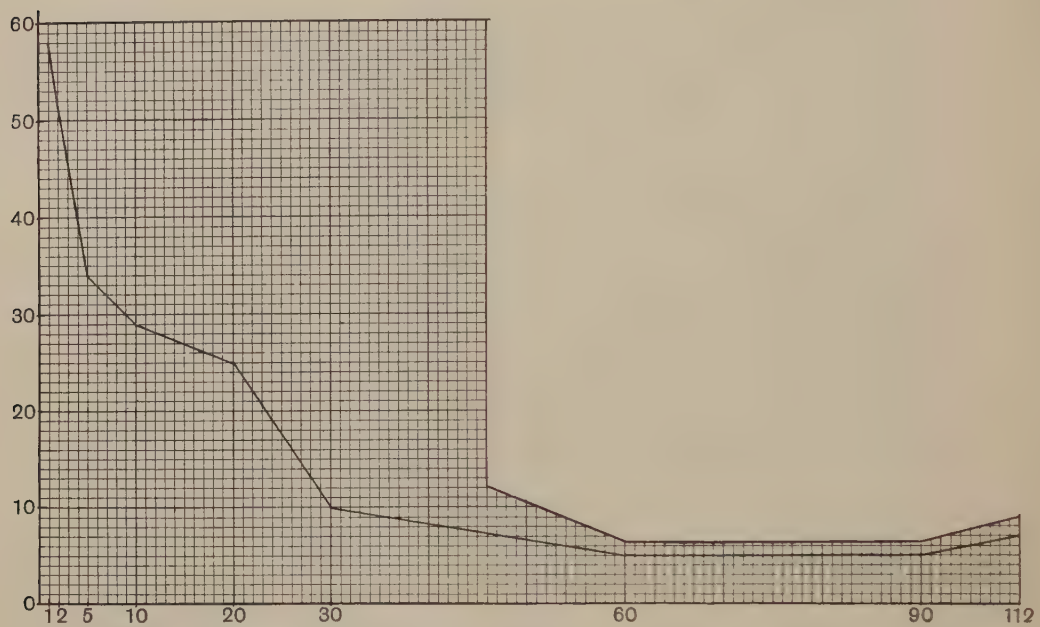

Fig. 5. Graph showing distribution of plants infected by the Swede Midge (Brood I) in a 1913 swede field adjacent to a 1912 swede field.
The rows are numbered from the side of the field next to the 1912 field.

is usually high. But in larger fields so situated the attack is to a large extent concentrated on one side and the field as a whole is not so badly attacked.

(*b*) *Evidence that flies of the later broods do not always remain in the field in which they have spent the larval and pupal periods.*

Before discussing various points which follow it is important to know whether any appreciable number of flies of the later broods leave the field in which they have spent the earlier stages of their life-history.

It is obvious that the flies of the first brood have to move a greater or less distance to find swedes as it is extremely unusual for swedes to be grown on the same ground in two consecutive years. And I obtained evidence in 1913 that many flies of the second brood did lay their eggs in fields in which they had not spent the pupal stage. Many fields, chiefly late sown ones which missed the first brood altogether, were attacked by the second brood, often having a percentage of infected

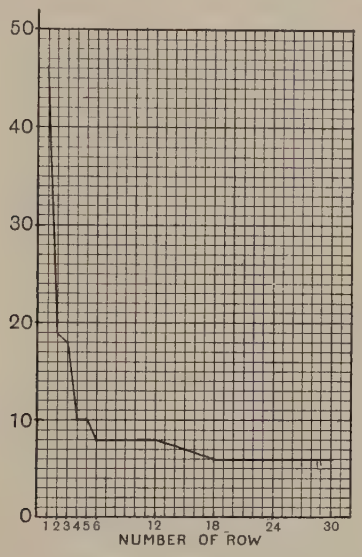

Fig. 6. Graph showing distribution of plants infected by Brood II of the Swede Midge in a late sown 1913 swede field which was not attacked by Brood I, situated near to an earlier sown 1913 swede field which was attacked by Brood I.

The rows are numbered from the side of the field near to the earlier sown field.

plants quite high for the year 1913. The following are a few examples taken from a considerable number of such cases:

		Date of sowing	Brood I % CL	Brood II % CL
F.	District I	May, last week	0	3
G.	„ IX	June, first week	0	9
H.	„ IV	June, second week	0	3
I.	„ VII	June, second week	0	10
J.	„ VII	June, third week	0	20

Clear evidence of the actual movement of flies from one particular field, comparatively early sown, to a later sown field, which missed the first brood altogether, is given in the graph, Fig. 6. Here the % CL

(Brood II) is plotted for various rows in Field K at Driffield, sown June, last week, which was next to Field L, sown June, first week, which was attacked by the first brood of the Midge. The two lots of swedes were separated by a hedge and about thirty yards of barley. The graph shows a very marked drop in the percentage of plants attacked in Field K as the distance from Field L increased.

In 1914 the evidence of such movement was not so clear.

(*c*) *Factors determining the Swede Midge index in different districts.*

The indices for each district in each survey are given in Tables 2 and 3, and plotted on Charts 1 to 5. On the charts for the Brood II and Brood III surveys I have also shown in which districts the index exceeded the index of the previous survey and in which districts the index was not greater. In Chart 5 the indices are the highest and the differences between the various districts are greatest.

The two factors which seem to be most important are:

(1) Average date of sowing,
(2) Distance from the sea.

(1) Average date of sowing.

In the northern part of my East Riding area, as I have shown (p. 88), the Midge is more numerous than in the southern part, and a glance at the charts will emphasize this fact. Mr Henry Ullyott, C.C., farmer and agricultural valuer, has kindly provided me with what in his experience are the average sowing dates for these two parts of my area. For each of the districts in the northern part the estimate which he gives is the last week in May; for all those in the southern part, the first week in June. In my survey work I examined fields just as I came to them, and the numbers of May sown and June sown fields which I visited in the northern and southern parts are as follows:

	1913		1914	
	Number of May sown fields	Number of June sown fields	Number of May sown fields	Number of June sown fields
Northern part	52	23	69	11
Southern part	27	49	55	54

In 1914, Mr Ullyott tells me, sowing was earlier than usual throughout the East Riding area. At Garforth, where the Midge is more numerous than in the East Riding, sowing is earlier. The average date there is the third week of May. Neither in 1913 nor 1914 did I come across any fields sown later than the last week in May.

I have already shown that May sown fields are worse attacked than

June sown fields, so that the larger number of June sown fields in the late sowing districts helps to keep the indices low in those districts, but a glance at Tables 2 and 3 will show that the average numbers of attacked plants for May sown fields in the early sowing districts are higher than for May sown fields in late sowing districts.

There is no evidence of any brood of the Midge in Yorkshire earlier than the first brood which attacks swedes. The question at once presents itself, therefore, what is it which brings to a close the long winter's rest? It cannot be mere lapse of time, for if it were we should never find an interval between two broods of one summer during which flies are relatively scarce or not to be found at all. During the Christmas and Easter terms of 1913—14 and the Christmas term of 1914 I have kept maggots in sand or soil indoors at average temperatures of from 55° F. to 70° F., and have reared a number of flies from them; but from maggots kept out of doors under the same moisture conditions, I have not reared a single fly. This suggests that temperature is an important factor, if not the only one, in bringing about the emergence of the first brood of midges. Now from observations in the field in 1911 on the length of time during which the flies of a brood could be found, it seems unlikely that the average lifetime of the fly is more than a week. We are thus able to understand the importance to the Midge of the average sowing date of swedes.

Swedes grow faster in the later sowing parts of my East Riding area than in the earlier sowing parts, doubtless owing to slightly higher temperature. Moreover, throughout my East Riding area, earlier sowing than is actually the practice is prevented by the fear of Leaf Mildew which attacks early sown swedes more than late sown ones. This mildew may therefore be looked upon as, indirectly, a limiting factor of the Swede Midge.

It is interesting to find that in the United States the chief way of combating that dangerous Cecidomyiid enemy of wheat, the Hessian Fly, is deliberate late sowing.

There may, of course, be other factors contributing to the greater success of the Midge in the early sowing districts. One factor may be the proportion of land under swedes. This is somewhat higher on the Wolds than in Holderness, because a four course rotation is the rule on the Wolds, while a five course one is the practice in Holderness. In Holderness, too, more mangels are grown as a root crop and the proportion of grass land is larger. Soil differences may be another factor. But it is especially notable that the Little Weighton wold district

(District XI), where the average sowing date is the first week of June, has very low indices.

I am also aware that the presence or absence of the cresses *Nasturtium palustre* and *N. silvestre* may prove to be a factor of importance. J. F. Robinson[1] records these cresses from the Holderness part of my area, but not from the Wolds. This point requires investigation.

(2) Distance from the sea.

The Swede Midge is less numerous in seaside districts than in inland districts. I have shown this to be the case on p. 88 and the charts show it quite clearly. The frequent failure of the Midge to increase in number from one brood to another in these seaside districts may be noted.

An explanation of these low indices was suggested to me by an accidental observation at Garforth early in August 1912. A bowl of water happened to be standing about a foot and a half from the ground near to a swede field. Within five minutes of each other two flies got on to the surface of the water in this bowl. As soon as a Swede Midge fly drops on to a surface of water it is helpless. This suggested to me that in districts near the sea the Swede Midge indices are kept low by the drowning of flies in the sea. Flies of the first brood must move greater or less distances if they are to find swedes. I have shown that some flies of the later broods leave the fields in which they have spent the earlier stages of their life-history, so that the later broods could suffer as well as the first. The midges are by no means strong fliers for if one is liberated in quite a gentle breeze it appears to be blown helpless by the wind. Two fields in the Flamborough district (District III) in 1913 do, I think, throw light on the problem. They were comparatively small plots next to ground which had grown swedes in 1912. The sowing dates and percentages of plants attacked by Brood I were:

M. May, 3rd week. 10 %.
N. May, 3rd week. 6 %.

In the other fields in the district the highest percentage of attacked plants was 2·5 %, and the average 0·7 %. These figures suggest that when the flies have only a short journey to make near the sea many of them accomplish it safely, but that when they have to move further in search of swedes many of them are lost in the sea.

I have examined wind records kept at Bridlington for the period June 1st to Sept. 30th of 1913 and 1914. In 1913 the frequency and

[1] *The Flora of the East Riding of Yorkshire*, 1902.

strength of winds which would tend to blow midges into the sea and also from inland to seaside districts were about equal with the frequency and strength of winds which would tend to blow flies from seaside to inland districts. In 1914 the seaward winds were about twice as frequent and of somewhat greater force than those blowing off the sea.

It has been objected that even if many flies were drowned in the sea their places would be taken by flies which moved from inland districts; such movement probably does take place. A glance at the five charts will show, however, that since the time of the first brood of 1913, when the Midge was reduced almost to a uniform low level in the East Riding and at Garforth, the tendency has been for differences between the indices of the richer and the poorer districts to become greater. This is in spite of whatever migration there may have been from one district to another.

(*d*) *Seasonal variations in the abundance of the Swede Midge.*

The fluctuations in abundance of the Midge from the first brood of 1912 to the third brood of 1914 are shown in Table 1, and for 1913 and 1914 have been summarized on p. 88.

The summer of 1911 was exceptionally hot; that of 1912 exceptionally wet and cold; that of 1913 was unusually dry with the temperature about normal; and in the summer of 1914 the rainfall was about normal and the temperature a little above the average.

In the summer of 1911 the Midge increased enormously. The first brood of 1912 was a very large one, but at the end of the summer maggots were very scarce, and the first brood of 1913 was a very small one. The Midge made slow progress in 1913, but rather greater progress in 1914.

Doubtless there is an important relation between the weather and the prosperity of the Midge. In Table 1 it will be seen that the progress made by the Midge in a summer varies roughly with the average mean air temperature or with the number of hours of bright sunshine. With the rainfall figures there is no such clear parallel. Rain probably is harmful to the Midge by washing eggs off the plants and by interfering with flight and egg laying. In hot weather and in the warmer parts of the day the flies are more active than when the temperature is lower. In a hot summer such as 1911 the time taken by the life cycle is shorter than in a cooler summer such as 1913. In some preliminary experiments the time elapsing between the going down into the soil of maggots and the emergence of the flies was found to be shorter at comparatively high than at comparatively low temperature. But I do not think it profitable to discuss further the effect of weather upon the Midge until I have

investigated the relation between the Midge and a Proctotrypid parasite which I reared from some maggots in the summer of 1914, and have searched for further parasites. For it is quite possible that the slaughter of 1912 was largely performed by parasites, and would have taken place even if that summer had been as fine as the preceding one.

It is recorded for the Hessian Fly that a summer in which the fly multiplies greatly is often followed by one in which it is extremely reduced, and this reduction is attributed to parasites. P. Marchal records[1] that in Vendée in 1894 the Hessian Fly increased greatly during the summer, but that it was very reduced in numbers in 1895. His explanation is that this was largely due to parasites which had also increased in 1894, though they commenced to hibernate earlier than the Hessian Fly. The flies of the last brood of 1894 were thus able to lay their eggs without the larvae hatching from those eggs being attacked by the parasite. But in 1895 the parasites hatched out in such numbers that they were able to kill a very large proportion of the maggots of the first brood of the Hessian Fly. A similar thing may have happened to the Swede Midge in 1912. I am therefore aware that the relation between the Midge and the weather is very likely not so simple or direct as it may appear to be from a glance at Table 1.

(*e*) *Other conclusions drawn from a comparison of the figures collected in the five surveys.*

In 1912 although I did not carry out a systematic Brood-I survey I was able to see that there were large differences between the percentages of infected plants which I found in different districts. But in 1913 the first brood was reduced almost to the same dead level over all the country in which I worked, at Garforth and in the East Riding. This general levelling may have been due to two causes:

(1) The killing of a larger proportion of the Midge individuals in the more densely populated districts.

This quite possibly happened, though I have no evidence on the point.

(2) The migration of flies from more densely to less densely populated districts.

Such movements of flies probably did take place, for I have shown that while flies of the first brood must move greater or less distances if they are to find swedes, flies of the later broods also sometimes move

[1] "Les Cécidomyies des Céréales et leurs parasites," *Ann. Soc. Entom. France*, 1897, Vol. LXVI, p. 12.

away from the fields in which they have spent the pupal stage. So one would naturally expect more flies to move from rich to poor districts than from poor to rich districts, though I have already pointed out that any such migration which may take place does not occur to an extent large enough to prevent there being marked differences between the indices of different districts. A comparison of Charts 1 and 5 will emphasize this point.

If, however, it is the case that more flies move from the Holderness Inland district to the Holderness Coast district, one conclusion seems to follow. In not a single survey is the average percentage of attacked plants in the Holderness Coast district as high as 1 %. From 1913 Brood I to 1914 Brood III the Garforth index has increased from 2 to 76, and the Midge has made considerable though less marked progress in other districts, but in the Holderness Coast district it has made absolutely no progress. I therefore suggest that were it not for the continual immigration of flies the Midge would soon cease to exist in this district. This may be the case too in the Flamborough district. And probably the Filey and Bempton figures are kept higher by immigration than they would be if those seaside districts were separated from inland districts by an impassable barrier. And it may be that the reason for the Little Weighton indices being lower than those of the Bainton and Holderness Inland districts is that those two districts receive more support from neighbouring earlier sowing districts.

One fact, though it scarcely requires census work to demonstrate it, is brought out quite clearly by a comparison of the figures for the different broods. One female fly contains about a hundred eggs, but the charts show that the Midge made but slow progress both in 1913 and 1914. There must therefore be a great mortality at some stage or stages of the life-history even in fine weather. This is doubtless due to a variety of causes.

In seaside districts, I believe, many flies are lost in the sea, but inland probably a large number of flies fail to find swedes—perhaps fail to find a mate—and die without leaving offspring.

There is probably a considerable mortality in the soil. In experiments I have only reared about 30 % of the maggots put into soil, even with plenty of moisture. And maggots die if they get too dry. In one experiment 100 maggots were put into dry soil from the surface of a garden bed and kept at a temperature at which maggots with plenty of moisture will pupate successfully. In this experiment no flies

appeared and on searching the soil four months later I found 99 dead maggots. Lack of moisture in the soil is therefore a probable source of loss.

The hoeing up in singling of plants harbouring small maggots must surely be a source of loss, though maggots are able to extricate themselves from plants left lying on soil, and quite small maggots are able to pupate successfully, though, of course, they turn into small flies. But I find that the proportion of small maggots which pupate successfully is lower than the proportion of larger maggots, and that such small flies contain fewer eggs than larger flies although the eggs in both are about the same size.

The Midge has some enemies. An Empid fly which sucks the juices from the flies was common at Garforth in June 1914. Some Midges are accounted for by small spiders. And in September 1914 in some experiments I reared a number of Proctotrypid flies from Swede Midge maggots.

In 1912 in the East Riding the competition of the Turnip Flea Beetle, *Phyllotreta nemorum*, was a matter of some importance. The Flea Beetle attacks the seed leaves of swede plants while the Midge does not attack them until they get into rough leaf. In 1912 the Flea Beetle was rampant in the East Riding and often made it necessary to sow two or even three times.

One more conclusion may be drawn from a comparison of the figures for the different years. If it is not already possible it probably would be possible after a few years further work to foretell, roughly, before the first brood makes its appearance, the sort of attack which may be expected. In 1911 the Midge increased enormously in number and the first brood of 1912 was a very large one. In 1912 the numbers of the Midge were greatly reduced and the first brood of 1913 was a very small one. In 1913 the Midge increased in number, but did not make much progress, and the first brood of 1914 was by no means numerous. If it is the weather which is the chief factor responsible for these ups and downs of the Midge, then weather statistics alone for a summer, without actual observations in the field, would probably be enough to enable one to make a more or less accurate prophecy of what sort of attack to expect. Unless winter weather is of much importance, and there has not been a severe winter since I first became interested in the Midge, it would be possible to make this prophecy six or eight months before the first brood appeared. I have already pointed out (p. 96), however, the possible complication which may be introduced

by parasites. As I have already remarked, it may be that the relation of the Midge to the weather is not so simple as it may at present appear to be.

VI. Summary of chief conclusions.

1. The Swede Midge was found to be present in 1912, 1913, and 1914 both at Garforth in the West Riding of Yorkshire and in all parts of the area in which I worked in the East Riding. At the same time it was possible to measure differences, sometimes very marked, in the relative numbers of the Midge at different places and at different times.

2. Conditions determining the percentage of plants attacked by Brood I of the Midge in a given field are:

(1) Date of sowing (p. 89),
(2) Distance from a swede field of the previous year, and
(3) Size of field (p. 89).

3. In an early sown swede field adjacent to an early sown swede field of the previous year a drop, often a very marked one, in the percentage of attacked plants could usually be plotted as the distance in that field from the swede field of the previous year increased (pp. 89, 90).

4. An appreciable number of flies of broods later than the first sometimes leave the field in which they have spent the larval and pupal periods, and lay their eggs in another field (p. 91).

5. Factors determining the Swede Midge index in a given district seem to be:

(1) Average date of sowing (p. 92).
(2) Distance from the sea (p. 94).

6. The prosperity of the Swede Midge appears to depend very largely on summer weather (p. 95). In this connection, however, the relation between the Midge and any parasites requires investigation. I have reared Proctotrypid flies from larvae of the Midge (p. 98).

7. Very likely the Midge would cease to exist in the Holderness Coast district if it were not for the immigration of flies into that district.

8. There is a great mortality at some stage or stages of the life-history.

9. In 1912 in the East Riding the Turnip Flea Beetle, *Phyllotreta nemorum*, was a competitor of some importance with the Midge.

APPENDIX.

1913 and 1914. Dates of surveys and tests of accuracy of surveys.

1913 *Brood I.*

July 9—31.
One field at Driffield repeated.
Survey, July 16 and 18, in two rows, 354 infected plants.
Repeated, Aug. 6, in same two rows, 374 infected plants.

1913 *Brood II.*

Aug. 12—Sept. 12.
Six fields repeated in Districts III and IV.
Survey, Aug. 14—20, % CL, 0·25, 2·5, 0·7, 4·5, 3·5, 1·0.
Repeated, Sept. 9, fields in same order, % CL, 0·25, 2·5, 1·25, 5·0 4·0, 2·0.
This I believe to be the least accurate of my five surveys.

1914 *Brood I.*

July 7—26.
Two fields repeated.
A, in District VII. Survey, July 10, in three rows, 241 infected plants.
Repeated July 26, in same three rows, 214 infected plants.
B, in District IX. Survey proper, July 15, in two rows, 95 infected plants.
Repeated, July 26, in same two rows, 95 infected plants.

1914 *Brood II.*

Aug. 17—28.
Seven fields repeated in District V.
Survey, Aug. 18, % CL, 6, 9, 10, 14, 13, 10, 8.
Repeated, Aug. 28 and 29, fields in same order, % CL, 9, 12, 10, 14, 8, 11, 9.

1914 *Brood III.*

Sept. 22—Oct. 4.
Eight fields repeated in District V.
Survey, Sept. 22, % CL, 23, 15, 15, 21, 12, 19, 13, 16.
Repeated, Oct. 4, fields in same order, in same rows as in survey proper, % CL, 18, 21, 13, 19, 10, 18, 13, 13.

There was no danger of more than very slight inaccuracy in each of the Brood I surveys from more Brood I flies coming out after the survey began, for each year the Brood I maggots had to a large extent left the plants before the Brood I survey began.

In the Brood II test fields, in both years, neighbouring rows to those examined in the survey were selected, not the actual rows examined in the survey, because in examining a plant at the time when infected leaves were small before deciding whether it was attacked or not I had sometimes destroyed the signs of attack. In the other surveys I was able to avoid doing this in the fields which I afterwards repeated as tests.

TABLE 1.

Meteorological data for the period June 1st to Sept. 30th for each of the years 1911, 1912, 1913, 1914, *and average percentages of plants attacked by the Swede Midge* (% *CL*). *The figures given below are calculated from figures published in the monthly weather reports of the Meteorological office. The figures given for the East Riding are the mean of figures calculated for Scarborough and Hull.*

	Year	Average Mean Air Temperature, °F.	Bright sunshine, hours	Rainfall, inches	Number of days on which rain fell (out of 122 days)	Average % CL (Brood I)	Average % CL (Brood II)	Average % CL (Brood III)
East Riding	1911	60·2	793	10·58	51	—	—	—
	1912	56·2	335	13·72	73	38*	—	—
	1913	57·7	520	3·40	40	1·2	2·4	—
	1914	58·7	610	9·73	44	2·3	3·2	6·6
Garforth	1911	58·2	783	8·26	46	—	—	—
	1912	55·4	380	14·16	67	94†	—	—
	1913	56·3	512	4·05	50	1·9	—	—
	1914	57·1	688	8·77	47	22	32	76

* 27 fields were examined between July 16th and 29th.

† 7 fields were examined on August 2nd.

TABLE 2.

1913. *Average percentages of attacked plants and Swede Midge indices for each district for the two surveys. The averages are given for May sown fields, June sown fields, and for all fields irrespective of sowing date.*

	District	Brood I							Brood II							
		May sown		June sown		All fields		Swede Midge index	May sown		June sown		All fields		Swede Midge index	Increase or not over Brood I index
		Number of fields	Average % CL	Number of fields	Average % CL	Number of fields	Average % CL		Number of fields	Average % CL	Number of fields	Average % CL	Number of fields	Average % CL		
I.	Filey	8	1·7	3	1·4	11	1·7	2	8	3·1	3	2·8	11	3·0	3	Yes
II.	Bempton.....	9	2·4	2	0·2	11	2·0	2	9	2·8	2	0·7	11	2·4	3	Yes
III.	Flamborough ...	10	0·8	1	0·2	11	0·7	1	10	0·4	1	0·2	11	0·4	1	No
IV.	Dale Towns	3	2·2	8	0·5	11	0·9	1	3	2·7	8	2·3	11	2·4	3	Yes
V.	High Wolds	14	0·9	6	0·1	20	0·7	1	14	3·6	6	2·7	20	3·3	4	Yes
VI.	Reighton.....	8	3·3	3	0·5	11	2·5	3	8	4·5	3	3·0	11	4·1	5	Yes
VII.	Low Wolds	11	2·8	16	0·5	27	1·5	2	11	5·8	16	5·0	27	5·5	6	Yes
VIII.	Bainton	—	—	4	1·7	4	1·7	2	—	—	4	1·6	4	1·6	2	No
IX.	Holderness Inland	7	1·3	10	0·2	17	0·7	1	7	2·6	10	2·4	17	2·5	3	Yes
X.	Holderness Coast.	4	1·3	16	0·1	20	0·4	1	4	0·5	16	0·5	20	0·5	1	No
XI.	Little Weighton..	5	0·5	3	0·1	8	0·4	1	5	0·7	3	0·1	8	0·5	1	No
Averages (East Riding)		—	1·7	—	0·5	—	1·2	—	—	2·7	—	1·9	—	2·4	—	—
XII.	Garforth	12	1·9	—	—	12	1·9	2	—	—	—	—	—	—	?	—

TABLE 3.

1914. *Average percentages of attacked plants and Swede Midge indices for each district for the three surveys. The averages are given for May sown fields, June sown fields, and for all fields irrespective of sowing date.*

		Brood I							Brood II								Brood III							
		May sown		June sown		All fields			May sown		June sown		All fields				May sown		June sown		All fields			
	District	Number of fields	Average % CL	Number of fields	Average % CL	Number of fields	Average % CL	Swede Midge index	Number of fields	Average % CL	Number of fields	Average % CL	Number of fields	Average % CL	Swede Midge index	Increase or not over Brood I index	Number of fields	Average % CL	Number of fields	Average % CL	Number of fields	Average % CL	Swede Midge index	Increase or not over Brood II index
I.	Filey	11	4·0	1	1·0	12	3·7	4	11	3·8	1	1·0	12	3·6	4	No	11	7·1	1	7·0	12	7·1	8	Yes
II.	Bempton	8	3·8	—	—	8	3·8	4	8	3·5	—	—	8	3·5	4	No	8	7·3	—	—	8	7·3	8	Yes
III.	Flambro'	9	0·6	2	0·5	11	0·5	1	9	1·2	2	2·0	11	1·2	2	Yes	9	1·9	2	1·0	11	1·7	2	No
IV.	Dale Towns	10	3·6	5	2·6	15	3·3	4	10	5·3	5	4·4	15	5·0	5	Yes	10	17·2	5	8·9	15	14·4	15	Yes
V.	High Wolds	21	3·0	3	1·0	24	2·7	3	21	5·9	3	1·7	24	5·4	6	Yes	21	12·0	3	4·0	24	11·0	11	Yes
VI.	Reighton	10	4·6	—	—	10	4·6	5	10	5·8	—	—	10	5·8	6	Yes	10	13·0	—	—	10	13·0	13	Yes
VII.	Low Wolds	19	3·5	8	1·2	27	2·8	3	19	4·8	8	2·3	27	4·0	4	Yes	19	8·5	8	2·7	27	6·8	7	Yes
VIII.	Bainton	5	1·7	4	1·6	9	1·7	2	5	3·3	4	2·8	9	3·1	4	Yes	5	6·1	4	3·3	9	4·8	5	Yes
IX.	Holderness Inland	21	1·9	23	1·1	44	1·5	2	21	2·9	23	2·0	44	2·4	3	Yes	21	6·3	23	2·8	44	4·5	5	Yes
X.	Holderness Coast	7	0·8	14	0·5	21	0·6	1	7	0·8	14	0·4	21	0·6	1	No	7	0·5	14	0·1	21	0·3	1	No
XI.	Little Weighton	3	0·5	5	0·3	8	0·4	1	3	0·7	5	1·0	8	0·9	1	No	3	1·0	5	1·3	8	1·2	2	Yes
	Averages (East Riding)	—	2·5	—	1·1	—	2·3	—	—	3·5	—	2·0	—	3·2	—	—	—	7·4	—	3·5	—	6·6	—	—
XII.	Garforth	12	22	—	—	12	22	22	12	32	—	—	12	32	32	Yes	12	76	—	—	12	76	76	Yes

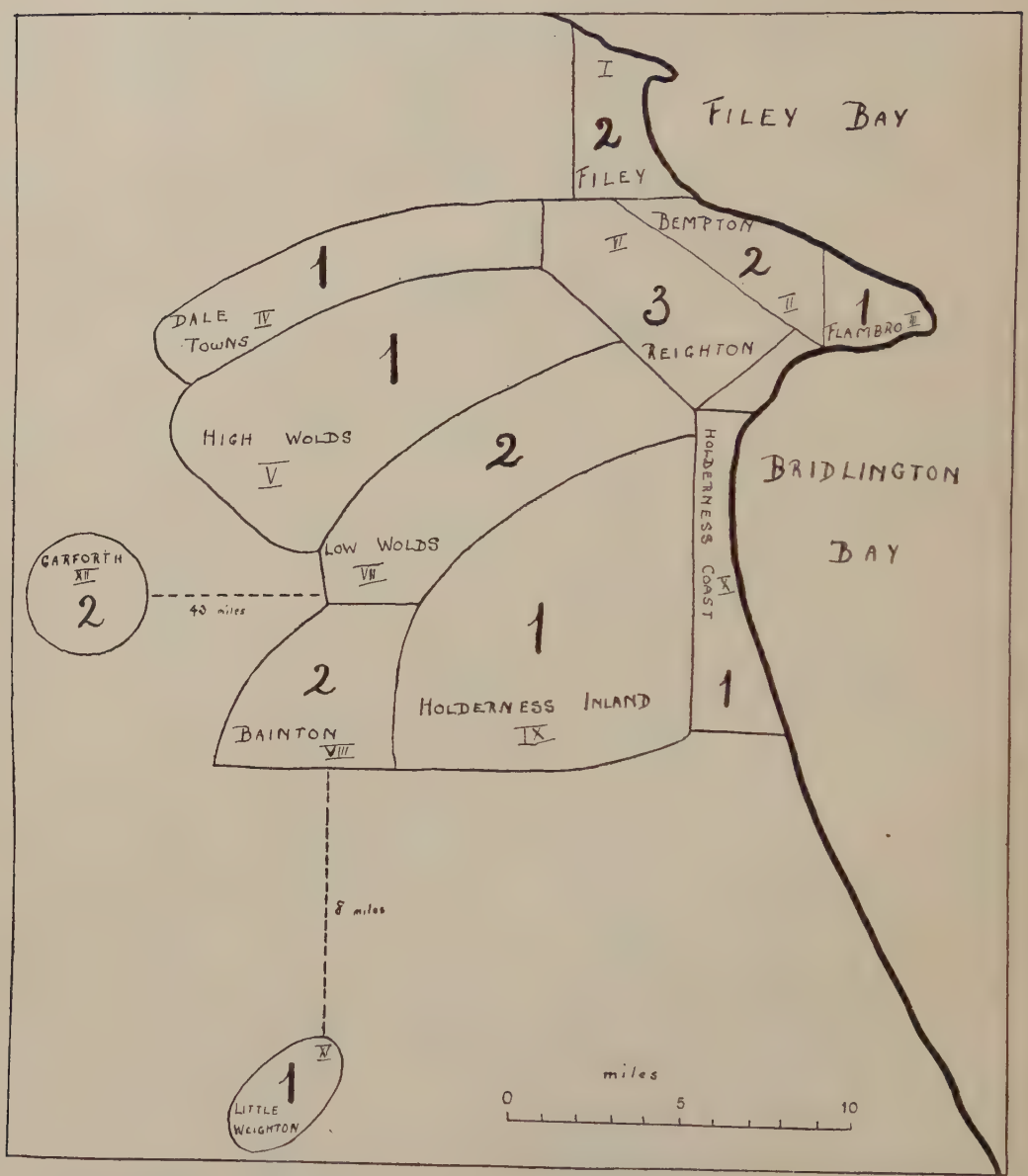

Chart 1. Showing for each district the relative density of Brood I of the Swede Midge in 1913.
Roman numeral = number of district.
Arabic numeral = index (Brood I).

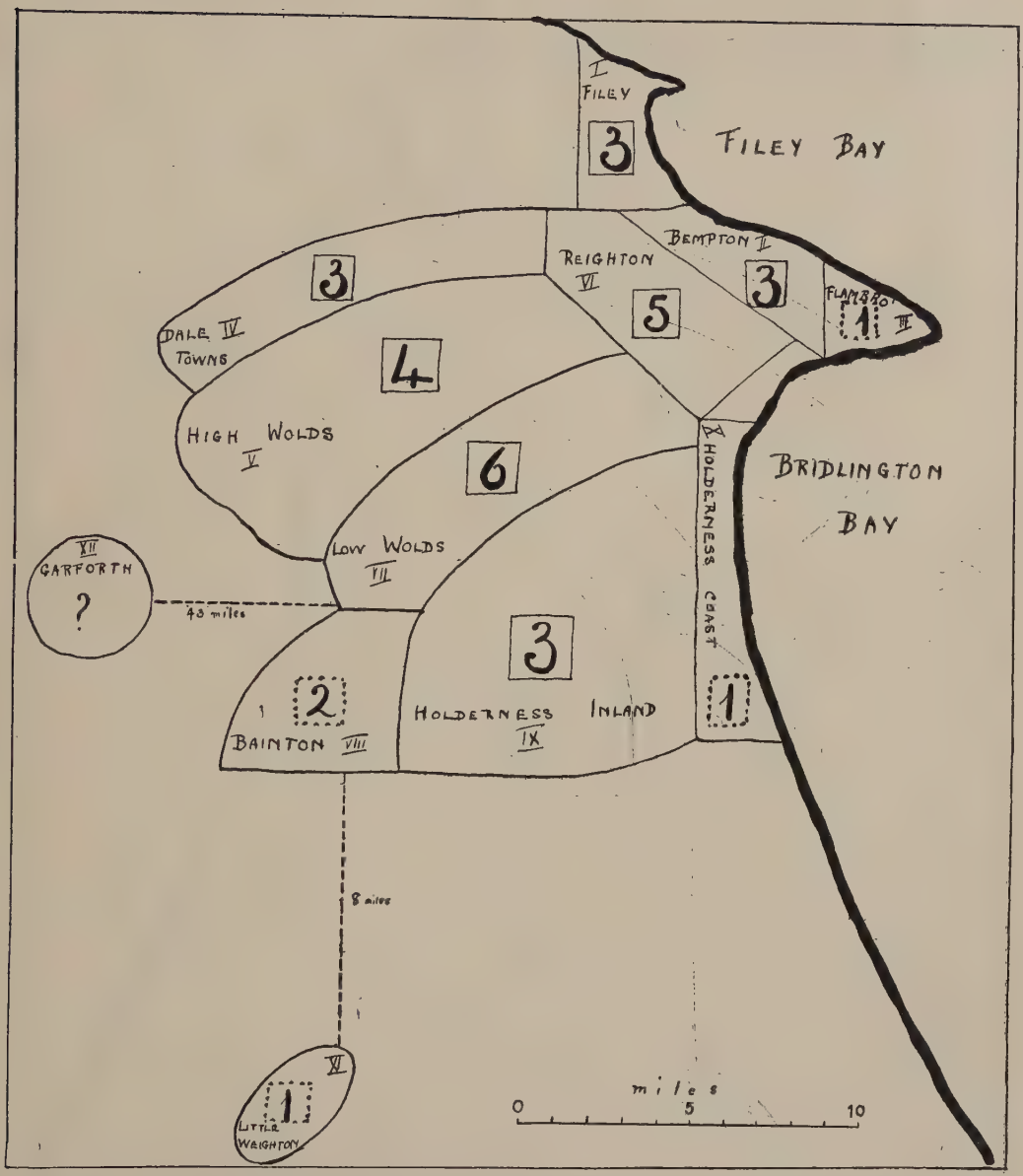

Chart 2. Showing for each district the relative density of Brood II of the Swede Midge in 1913.
Roman numeral=number of district.
Arabic numeral=index (Brood II).

A rectangle of unbroken lines round the index figure indicates that the Brood II index exceeds the Brood I index.

A rectangle of dotted lines round the index figure indicates that the Brood II index does not exceed the Brood I index.

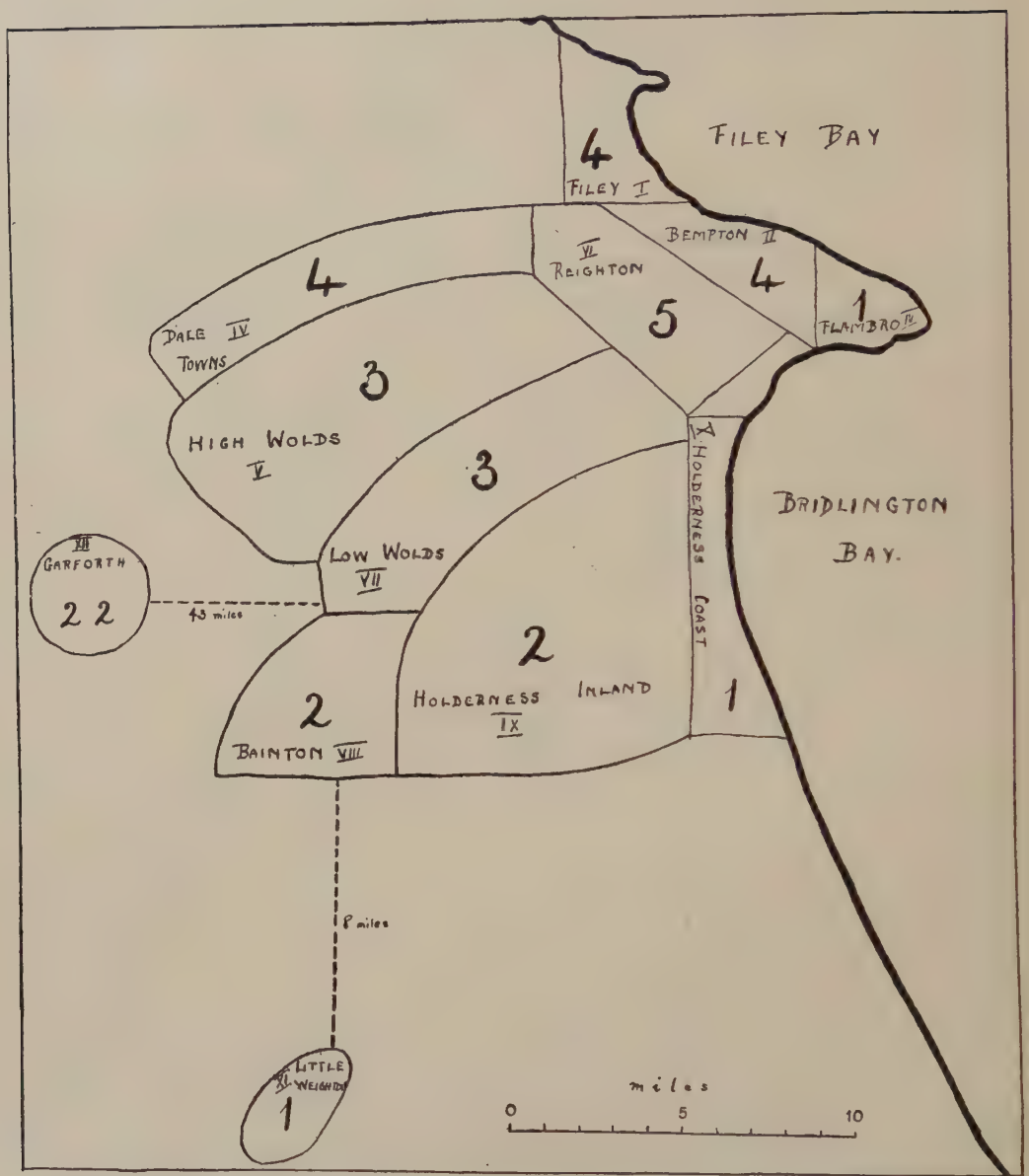

Chart 3. Showing for each district the relative density of Brood I of the Swede Midge in 1914.
Roman numeral = number of district.
Arabic numeral = index (Brood I).

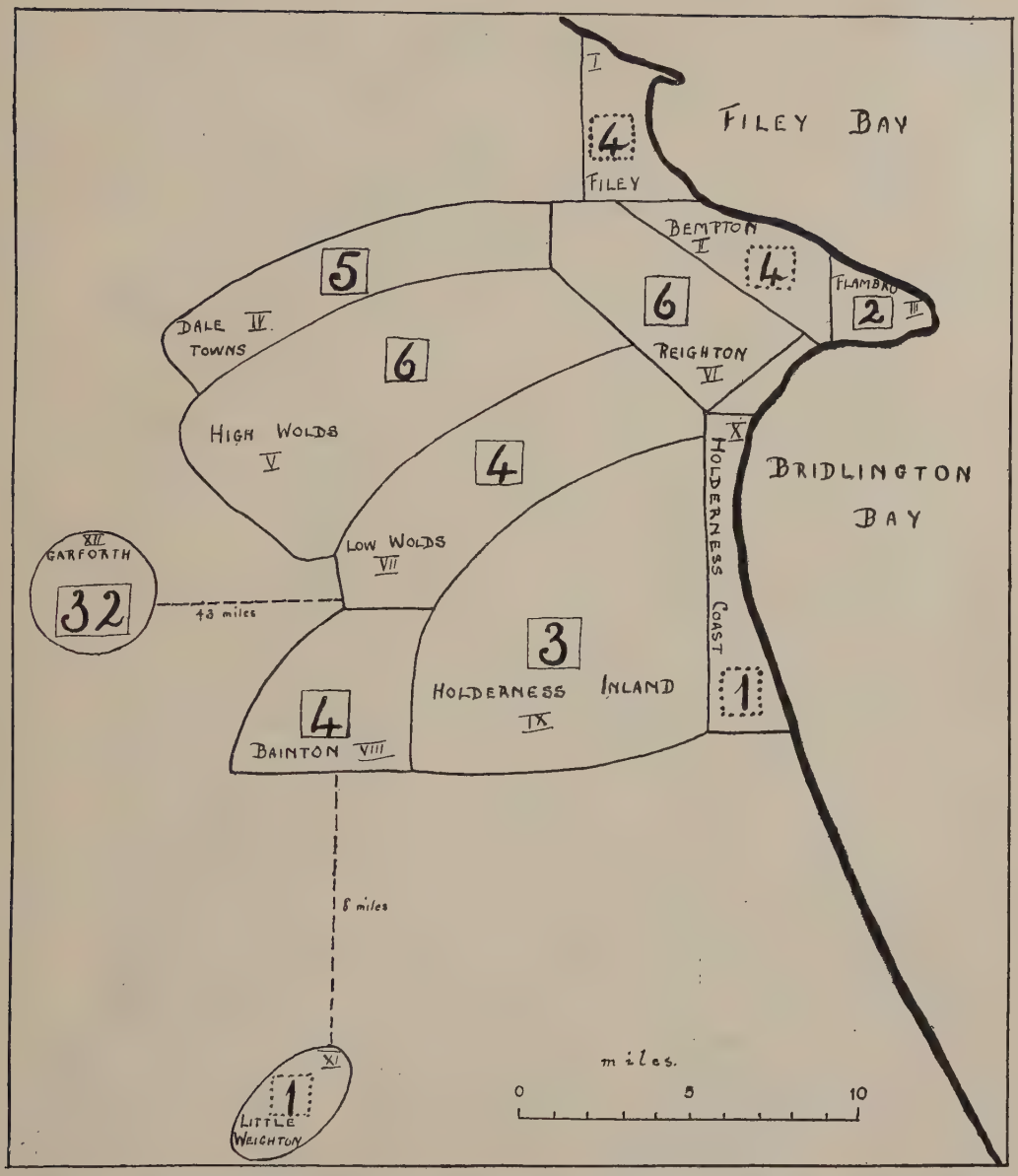

Chart 4. Showing for each district the relative density of Brood II of the Swede Midge in 1914.
Roman numeral = number of district.
Arabic numeral = index (Brood II).

A rectangle of unbroken lines round the index figure indicates that the Brood II index exceeds the Brood I index.

A rectangle of dotted lines round the index figure indicates that the Brood II index does not exceed the Brood I index.

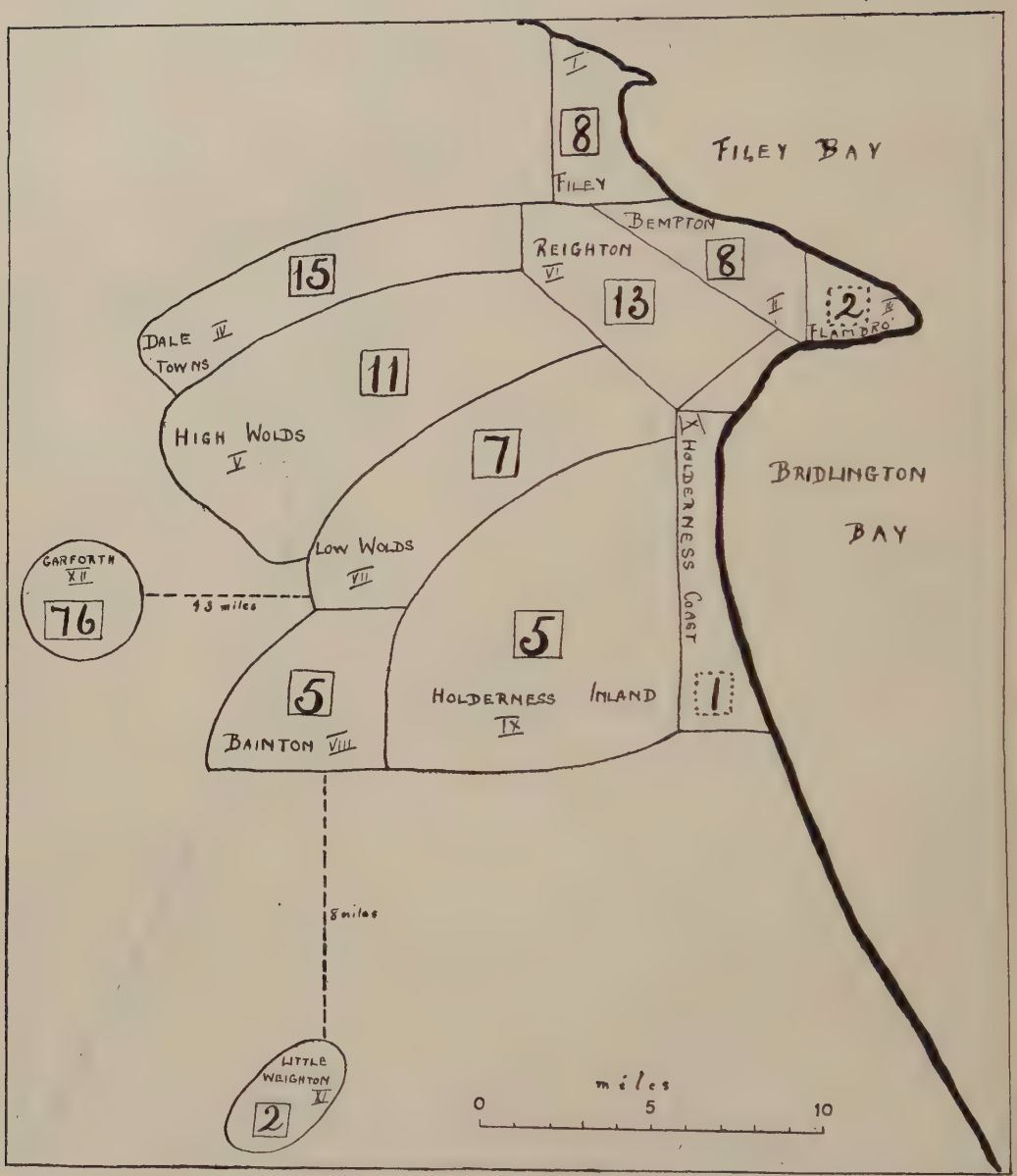

Chart 5. Showing for each district the relative density of Brood III of the Swede Midge in 1914.
Roman numeral = number of district.
Arabic numeral = index (Brood III).

A rectangle of unbroken lines round the index figure indicates that the Brood III index exceeds the Brood II index.

A rectangle of dotted lines round the index figure indicates that the Brood III index does not exceed the Brood II index.

THE OCCURRENCE OF FUNGI ON *ALEURODES VAPORARIORUM* IN BRITAIN.

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In 1902 H. M. Lefroy[1] reported the presence of a fungus on the brown shield scale (*Saissetia hemisphaerica*) in the West Indies which was subsequently identified as *Cephalosporium Lecanii* discovered by Zimmermann[2] infesting *Lecanium viride* (green bug) on the coffee plant in Java (1898). Both Zimmermann, and Parkin[3] who described *C. Lecanii* in greater detail (1906), ascribe to the fungus a parasitic habit. It was enrolled among the ranks of entomogenous fungi and has been used on a considerable scale to combat scale insects in the West Indies, Florida and elsewhere, and according to J. R. Bovell[4], F. W. South[5], G. G. Auchinleck[6] (citrus trees in Dominica and Grenada), G. E. Bodkin[7] (British Guiana), and W. Nowell[8] (Grenada, Dominica, Antigua) with some success.

Professor Lefroy in December, 1914, drew my attention to a considerable quantity of fungus on the leaves of Centropogon, at Wisley, also badly infested, for experimental purposes, with the nymph-form of *Aleurodes vaporariorum* (white fly). The upper surface of the leaves was covered with a sooty mould (*Capnodium*?): the under surface, infested with a quantity of eggs, nymphs and a few emerging and complete imagos, was covered with mycelial tufts of *Cladosporium* and wefts of *Cephalosporium*.

[1] H. M. Lefroy in *W. I. Bull.* III. (1902), 240, 295.

[2] Zimmermann; *Over eene Schimmel epidemie der Groene Luizen.* Buitenzorg, Java, 1898.

[3] J. Parkin in *Ann. Roy. Bot. Gard. Peradeniya,* III. Pt. 1 (1906), 41.

[4] J. R. Bovell in *W. I. Bull.* XII. No. 4 (1912), 399–402.

[5] F. W. South in *W. I. Bull.* XI. No. 1 (1911), 1–30, and *W. I. Bull.* XII. No. 2 (1912).

[6] G. G. Auchinleck in *W. I. Bull.* XII. No. 2 (1912).

[7] G. E. Bodkin in *Jour. Bd. Agric. Brit. Guiana,* V. No. 2 (Oct. 1911).

[8] W. Nowell in *W. I. Bull.* XIV. No. 3 (1914), 215.

The mode of occurrence of the *Cephalosporium* on the *Aleurodes* nymph agrees in every respect with Parkin's description and figure of *Cephalosporium Lecanii* on Lecanium, where the mycelial threads with their short capitate conidiophores are depicted radiating outwards from the body of the nymph, but in this case dead imagos invested by a weft of mycelium are also found.

Since this Cephalosporium, which will be described in detail elsewhere, proves to be quite distinct from Zimmermann's species and indeed from all other Cephalosporia, it has been named *Cephalosporium Lefroyi* (see *Gard. Chron.* March 13, 1915, p. 139).

Ever since P. H. Rolfs[1] demonstrated by orchard introductions that *Sphaerostilbe coccophila*, discovered by the Tulasnes in 1865, could be used to combat the San José scale, the use of entomogenous fungi has been extensively adopted in Florida where the conditions of temperature and moisture are conducive to the spread of fungi. Thus a list of six fungi parasitic upon *Aleurodes citri—Aschersonia aleyrodis* Webber, *Aschersonia flavo-citrina* P. Henn., *Verticillium heterocladum* Penz., *Sphaerostilbe coccophila* Tul., *Microcera* sp., and a sterile brown fungus, afterwards styled *Aegerita Webberi* (see *Mycologia*, II. No. 4, July, 1910)—is given by Howard S. Fawcett[2] (1908) of which *Sphaerostilbe coccophila* is stated to be rarely parasitic on this insect: a seventh is added by E. W. Berger[3] (1909), a species of *Sporotrichum*, which is stated to infect the adult and some larvae.

According to the Report of the Entomologist[4], Florida (1913), natural mortality of white fly is caused mostly by *Microcera*. On the other hand, Morrill and Back (1912)[5] report that mortality from unexplained causes proves to be the most important element of natural control. This mortality has never been taken into consideration in previous publications, and in the past no little confusion has existed owing to the failure to distinguish between the benefits derived from it and those from fungus parasites. *Microcera* is stated to be normally saprophytic.

We are therefore led to wonder to what extent the mortality amongst scale insects is really due to fungi. Both in Florida and the West

[1] P. H. Rolfs in *Fla. Agr. Exp. Sta. Bull.* 41 (1897).

[2] Howard S. Fawcett, *Fungi parasitic upon* Aleyrodes citri. Thesis Univ. State of Florida, June, 1908.

[3] E. W. Berger in *Fla. Agr. Exp. Sta. Bull.* 97 (Feb. 1909).

[4] Florida Agr. Exp. Sta. *Ann. Rept.* (1913), LIV–LVIII.

[5] A. W. Morrill and E. A. Back in U.S.A. Dept. Agr. Bur. of Ent. *Bull.* 102 (1912)

Indies the climatic conditions which favour the development of the fungi would appear to be inimical to the insects, as in Dominica; but where and when dry conditions exist at certain times of the year, as at St Kitts, few fungus enemies to insects are found.

Some observations made in the case of *Cephalosporium Lefroyi* on *Aleurodes vaporariorum* seem to be in agreement with the conclusions of Morrill and Back. They are as follows:

1. Dead imagos occur enclosed in a weft of Cephalosporium.
2. Feeble but not always old imagos are not in every case associated with Cephalosporium.
3. Crushed feeble imagos have not always yielded hyphae and spores.
4. Feeble imagos may bear portions of the mycelium of the fungus.
5. Dead, half-emerged imagos are often coloured orange and may or may not be associated with Cephalosporium.
6. There are large numbers of dead nymphs, some with Cephalosporium, some with Cladosporium, others without fungi.

We must carefully distinguish the case of the nymphal Aleurodes from that of the imago. The nymph practically remains *in situ* on the leaf. Fungi could live and grow on the honey-dew excretion caused by the insect and ultimately invest the nymph and close the tracheae, a method of action suggested by Giard[1] for *Cladosporium parasiticum* on *Polyphylla fullo*, and which may well happen in the case of the fungi under discussion.

Clearly the question of the parasitism of *Cephalosporium Lefroyi* and the other species of fungi associated with *Aleurodes vaporariorum* must remain open until the whole subject of fungi in relation to scale insects has been reinvestigated by improved methods and put on a more satisfactory basis.

[1] Giard in *Comptes Rendu*, cxii. 1518–21.